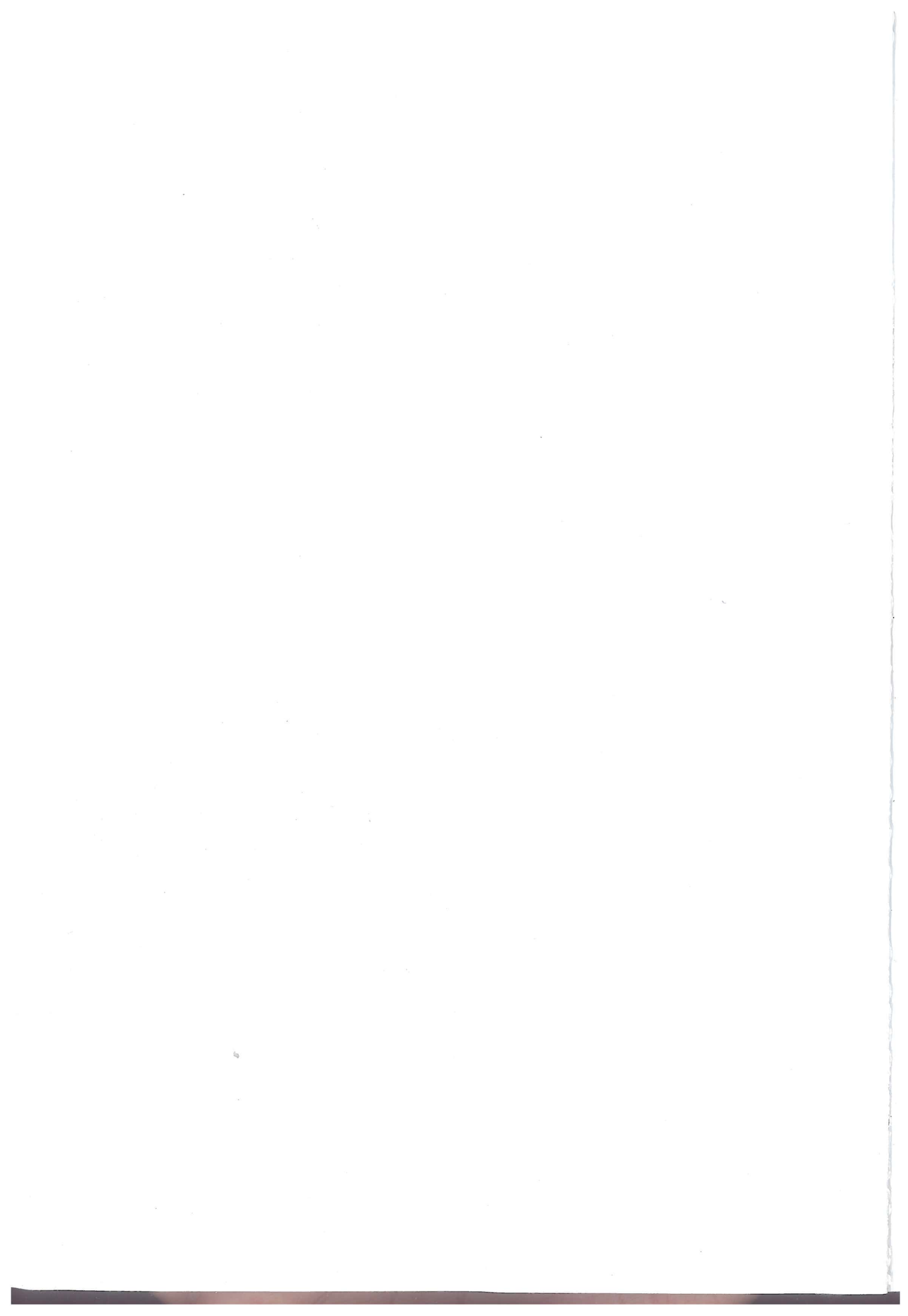


# ESSENTIAL MEDICAL MICROBIOLOGY and IMMUNOLOGY



Volume I





# P R E F A C E

*This book is intended to be a comprehensive and up to date guide to medical microbiology and immunology in the most reliable, attractive, illustrated manner for undergraduate medical students, as well as a guide for postgraduates preparing for higher degrees.*

*It is designed to cover the four aspects of medical microbiology and immunology (Bacteriology, Mycology, Virology, and Immunology) in four separate volumes in addition to a practical book, each navigates the reader to this ever expanding science.*

- Vol. I : General Microbiology (bacteriology, virology , mycology)
- Vol. II : Immunology
- Vol. III : Systematic Bacteriology
- Vol. IV : Systematic Virology, Systematic Mycology and Applied Microbiology

*In preparing this text, the primary objective of our panel was to supply the reader with a concise updated source reflecting the tremendous progress in our knowledge in the fascinating field of microbiology and immunology; it is our sincere hope that it can fulfil this goal.*

وَمَا زَادَنِي فَخْرًا وَتَبَاهَا

وَكُنْتُ بِإِخْمَصِ أَطَا الثَّرِيَا

دَخُلِي حَقَّ قَوْلِ الْبَيَادِ

وَأَنْ حَمِيْرَتِ أَحْمَدَ لِي نِيَا

The Authors



**ESSENTIAL  
MEDICAL MICROBIOLOGY  
AND IMMUNOLOGY**

**VOLUME I**

**General Microbiology  
(Bacteriology, Virology, Mycology)**

**Eighth Edition**

**By**

***Staff Members of  
Medical Microbiology and Immunology Department***

**Faculty of Medicine-Cairo University  
2018-2019**





Running nose معاهها 2 دور برد حاجه ١٧

انفلونزا الطيور مرض قاتل ينتقل باللمس فقط وليس مكاله فتح نزل المصوره مريضه الطيور

11 - الخنازير خيروس ضعيف ينتقل في الهواء

خيروس الطيور والخنازير يندمجوا مع بعضهما في جسم ايساسم يفعلوا فيروس جديد لسانه خلقش في العالم  
ينتقل في الهواء وينتقل في الهواء، زي الخنازير **CONTENTS** ويقتل اوكش صليار اسامه في اسن من الكشور

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١ - الخنازير دراسة الكائنات الصغيره → Microbiology

٢ - الكائنات الصغيره ١ فطريات  
٢ الكائنات → الخنازير ٢ فطريات  
خنازير ٢ فطريات  
خنازير ٢ فطريات

Gene therapy ← هيب اللعلاج به من 2025 مريضه مريضه الخنازير

MesoComial ← عدوى المستشفيات = الحقن الحقن الحقن

Eu, Pro = eu 58  
Bacteria = Pro "

Eu, Pro 59

Fungus, Virus, Bacteria " " - 2



## INTRODUCTION TO MICROORGANISMS

### ILOs:

By the end of this chapter the student should be able to:

- Recall system of microbial classification
- Identify the terms eukaryotes versus prokaryotes

حقيقي

أدنى

نواة

Microorganisms are generally unicellular i.e. the whole organism is one cell. In such cases, a single microbial cell performs all the functions required to maintain itself and propagate.

Microorganisms may be classified in the following large biological groups:

1. Algae. — الطحالب
2. Protozoa. — الأوليات → wasn't medical importance.
3. Slime moulds. — فطريات العفن → no critical importance.
4. Fungi. — الفطريات الخميرة (الباكتريا بالإنسان وتسبب أمراضا)
5. Bacteria. — البكتيريا
6. Archaeobacteria. — archae = أدنى عايشة في التربة ومارش لا زرع لينا
7. Viruses. — الفيروسات
8. Prions. — أصغر فيروس

Pro, eue ← المشترك بينهم = انهم True cells.

The algae (excluding the blue-green algae), the protozoa, slime moulds and fungi include the larger and more highly developed microorganisms; their cells have the same general type of structure and organization, described as *eukaryotes*, as that found in higher plants and animals. The bacteria, including organisms of the mycoplasma, rickettsia and chlamydia groups, together with the related blue-green algae, comprise the smaller microorganisms with a simpler form of cellular organization described as *prokaryotes*.

The most outstanding character of *eukaryotic* cells (*eu*=true; *karyote*=nucleus) is a distinct nucleus, surrounded by a membrane that separates it from the other contents of the cell. *Prokaryotic* cells (*pro*=before), on the other hand, do not contain a membrane-bound nucleus. Instead, their hereditary material is suspended in a portion of cytoplasm called nucleoid or nuclear region. They are also devoid of mitochondria and other membrane bound organelles.

شبيهة بالنواة

The viruses are one of the smallest infective agents; they have no cell structure. Viruses are obligate intracellular parasites; they require the biological machinery of a host cell for reproduction and survival. Even simpler are *viroids*, protein-free fragments of single-stranded circular RNA that cause disease in plants. *Prions* are described as infectious proteins devoid of nucleic acid.

Prions Cause Mad Cow disease مرض جنون البقر  
& cause infection in humans

Since the algae, slime moulds and archaeobacteria are not thought to contain species of medical or veterinary importance, they will not be considered further. Blue-green algae do not cause infection, but certain species produce potent toxins that may affect people or animals drinking polluted water.

**MCQs:**

- 1- Which of the following microorganisms has a nuclear membrane?
  - a- Viruses
  - b- Fungi
  - c- Prions
  - d- Bacteria
  - e- Viroids
  
- 2- Viruses have all the following characteristics **EXCEPT**:
  - a- They are one of the smallest infectious agents.
  - b- They have no cell structure.
  - c- They are obligate intracellular parasites.
  - d- They require the host biological machinery for their replication.
  - e- They are prokaryotic.
  
- 3- Prions:
  - a- Are single stranded circular RNA
  - b- Are devoid of proteins
  - c- Are infectious proteins devoid of nucleic acids
  - d- Are prokaryotic cells
  - e- Cause diseases in plants

# **GENERAL BACTERIOLOGY**





## BACTERIA: THEIR STRUCTURE AND ORGANIZATION

### **ILOs:**

**By the end of this chapter the student should be able to:**

- Recall different shapes and arrangements of bacterial cells
- Differentiate between the two main categories of bacteria based on Gram stain
- Describe intracytoplasmic structures of bacterial cells and outline their function
- Describe bacterial cell membrane and its function
- Recognize cell wall structure and function
- Define cell wall deficient bacteria
- Discuss structures outside cell wall with their functions
- Recall appendages structure and their functions
- Discuss bacterial spores and their medical importance
- Recognize causes of spore resistance
- Identify bacterial genus based on spore morphology

Bacteria were first discovered by **Leeuwenhoek 1674**. They are among the most widely distributed forms of life. They are found in air, water and soil. They are also found in or on the human body, animals and plants.

### **Bacterial Morphology**

Bacteria are differentiated into major categories, based on their morphological features such as shape, size, arrangement and staining characteristics.

#### **Bacterial Size**

Most bacteria range in size from 0.2-1.2  $\mu\text{m}$  in width and 0.4-14  $\mu\text{m}$  in length.

### Bacterial Shape and Arrangement

(A) **Cocci** (singular: coccus): are spherical organisms with a wide variety of arrangements, e.g.:

1. Diplococci: pairs of cells, e.g. *Neisseria*.
2. Irregular grape-like clusters, e.g. *staphylococci*.
3. Chains of four or more, e.g. *streptococci*.

(B) **Bacilli** (singular: bacillus=stick): are rod-shaped organisms. These cells may occur singly, in pairs or in chains. Some bacilli are short (*coccobacilli*), others are curved (*vibrios*).

(C) **Spiral bacteria**: are divided into two groups, spirilla which are rigid and spirochaetes that are flexible.

The arrangement of cells is determined by the planes of division. For example, the cocci that divide along a single plane produce diplococci or chains, e.g. *streptococci*, while those that divide on many planes produce clusters, e.g. *staphylococci*.

### Staining Characteristics

There are two kinds of stains: simple and differential. *Simple stains* employ a single dye like methylene blue, crystal violet or fuchsin. Cells and structures stained with them give the same colour. Therefore, they only reveal the characteristics of size, shape and arrangement. *Differential stains* require more than one dye, and distinguish between different types of bacteria by giving them different colours.

Gram's stain is the most important differential stain in clinical microbiology. It divides bacteria into Gram-positive (violet-staining) and Gram-negative (red staining). The second important stain is Ziehl-Neelsen stain. It is used to identify the acid-fast bacilli of the genus *Mycobacterium*.

### Bacterial Ultra-Structures and their Functions

All bacteria have a nucleoid, ribosomes and a cytoplasmic membrane. Most bacteria also have a cell wall and some are further enveloped by a capsule or slime layer. Some types of bacteria have also cytoplasmic inclusions and various appendages as flagella and pili. The final details of subcellular structures are best revealed by electron microscopy (Fig. 1).

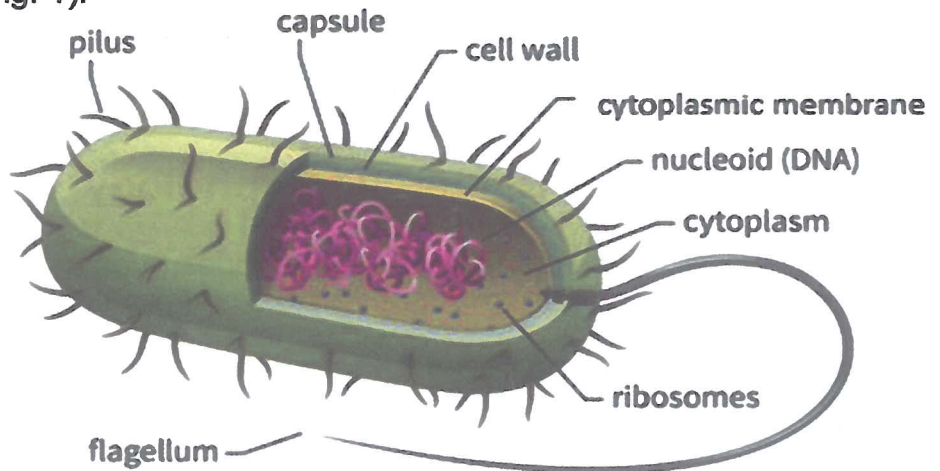


Fig. (1): Schematic presentation of a bacterial cell



## Cytoplasm

Few morphologically distinct components can be found within the cytoplasm:

**Nucleoid:** Genetic information of a bacterial cell is contained in a single circular molecule of double-stranded DNA, which constitutes the bacterial chromosome. It is 1 mm long and is packed into a supercoiled state inside the cell.

**Plasmids:** In many bacteria, additional genetic information is contained on plasmids which are small circular extrachromosomal DNA molecules that can replicate independently of the chromosome.

**Ribosomes:** They are the site of protein synthesis in the cell. Ribosomes consist of protein and RNA. Prokaryotic ribosomes have a sedimentation constant of 70S, smaller than the 80S ribosomes of eukaryotes. This difference makes bacterial ribosomes a selective target for antibiotic action.

**Inclusion granules:** These are granules of nutrient materials, usually phosphates, sulphur, carbohydrates and lipids. Energy reserves are usually stored as glycogen, starch or poly- $\beta$ -hydroxybutyrate. Phosphate is stored in metachromatic or volutin granules, which are used for synthesis of ATP.

**Mesosomes:** These are complex invagination of the cytoplasmic membrane. They are involved in cell division and sporulation. They also have a function analogous to the mitochondria in eukaryotes providing a membranous support for respiratory enzymes.

## Cytoplasmic Membrane

In bacteria, as in other cells, the protoplast is limited externally by a thin elastic cytoplasmic membrane. It is a phospholipid protein bilayer similar to that of eukaryotic cells except that, in bacteria, it lacks sterols. It has the following functions:

1. **Selective transport:** In bacteria, molecules move across the cytoplasmic membrane by simple diffusion, facilitated diffusion and active transport.
2. **Excretion of extracellular enzymes:**
  - a. Hydrolytic enzymes: which digest large food molecules into subunits small enough to penetrate the cytoplasmic membrane.
  - b. Enzymes used to destroy harmful chemicals, such as antibiotics, e.g. penicillin-degrading enzymes.
3. **Respiration:** The respiratory enzymes are located in the cytoplasmic membrane, which is thus a functional analogue of the mitochondria in eukaryotes.
4. **Cell wall biosynthesis:** The cytoplasmic membrane is the site of:
  - a. The enzymes of cell wall biosynthesis.
  - b. The carrier lipids on which the subunits of the cell wall are assembled.
5. **Reproduction:** A specific protein in the membrane attaches to the DNA and separates the duplicated chromosomes from each other. A septum forms by the cytoplasmic membrane to separate the cytoplasm of the two daughter cells.
6. **Chemotactic system:** Attractants and repellants bind to specific receptors in the cytoplasmic membrane and send signals to the cell's interior. The cell then responds to the surface message.



## Cell Wall

The bacterial cell wall is the structure that immediately surrounds the cytoplasmic membrane. It is 10-25 nm thick, strong and relatively rigid, though having some elasticity.

### Structure of the cell wall

The cell wall of bacteria is a complex structure. Its impressive strength is primarily due to peptidoglycan (synonym: murein or mucopeptide). Peptidoglycan is a complex polymer consisting of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) unique to bacteria. A set of identical tetrapeptide side chains are attached to NAM. Besides peptidoglycan, additional components in the cell wall divide bacteria into Gram-positive and Gram-negative (Fig. 2).

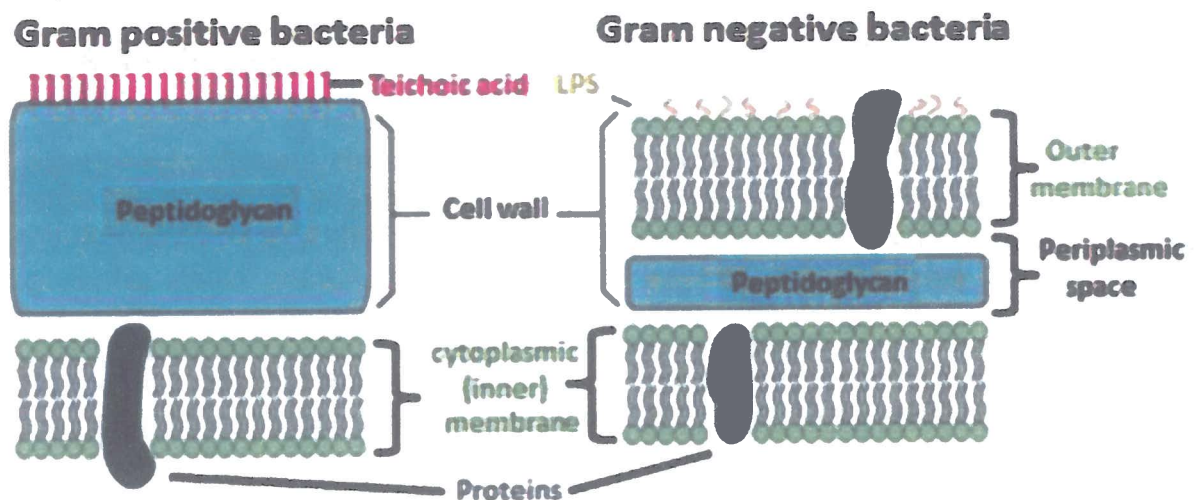


Fig. (2): Schematic presentation of Gram positive and Gram negative cell wall

### Gram-positive cell wall is composed of:

**Peptidoglycan:** There are as many as 40 sheets of peptidoglycan, comprising up to 50% of the cell wall material. Despite the thickness of peptidoglycan, chemicals can readily pass through.

**Teichoic acids:** They are the polymer of ribitol or glycerol phosphate. They are found in the cell wall of most Gram-positive bacteria. Teichoic acids and cell wall associated proteins are the major surface antigens of the Gram-positive bacteria.

### Gram-negative cell wall is composed of:

**Peptidoglycan:** It is much thinner, composed of only one or two sheets comprising 5-10% of the cell wall material.

**Outer membrane:** It is phospholipid protein bilayer present external to the peptidoglycan layer. The outer surface of the lipid bilayer is composed of molecules of lipopolysaccharide (LPS) which consists of a complex lipid called lipid A chemically linked to polysaccharides. Lipid A of the LPS forms the endotoxin of the Gram-negative bacteria, while polysaccharides are the outermost molecules of the cell wall and are major surface antigens of the Gram-negative bacterial cell (somatic or O antigen).

**Periplasmic space:** It is the space between the cytoplasmic and outer membranes. It contains the peptidoglycan layer and a gel-like solution of proteins.

**Functions of the cell wall**

1. It maintains the characteristic shape of the bacterium.
2. It supports the weak cytoplasmic membrane against the high internal osmotic pressure of the protoplasm (5-25 atm.).
3. It plays an important role in cell division.
4. It is responsible for the staining affinity of the organism.

**Wall deficient variants**

**a- Mycoplasma:** It is the only group of bacteria that exists naturally without cell wall. Mycoplasmas do not assume a defined recognizable shape, because they lack a rigid cell wall. These organisms are naturally resistant to cell wall inhibitors, such as penicillins and cephalosporins.

**b- L. Forms:** They are wall defective or wall deficient bacteria.

- "L" stands for Lister Institute in London, where they were first discovered.
- "L" forms may develop from cells that normally possess cell wall, when they are exposed to hydrolysis by lysozyme or by blocking peptidoglycan biosynthesis with antibiotics, such as penicillin, provided that they are present in an isotonic medium.
- Some L. forms resynthesize their walls once the inducing stimulus is removed. Others, however, permanently lose the capacity to produce a cell wall.
- L. forms may survive antibiotic therapy. Their reversion to the walled state can produce relapses of the overt infection.

**Capsule and Related Structures**

Many bacteria synthesize large amount of extracellular polymer that collects outside the cell wall to form an additional surface layer. This layer is formed only inside the host (*in-vivo*). With one known exception (the polypeptide capsule of *Bacillus anthracis*), the extracellular material is made of polysaccharides.

- **Capsule:** It is such a layer that adheres to the surface of the cell and forms a well- defined halo when differentially stained, to be resolved with the light microscope.
- **Slime layer:** It is a surface layer that is loosely distributed around the cell.
- **Glycocalyx:** It is a loose meshwork of polysaccharide fibrils extending outwards from the cell.

**Functions:**

1. It protects the cell wall against various kinds of antibacterial agents, e.g. bacteriophages, colicins, complement and lysozymes.
2. It protects the bacterial cell from phagocytosis. Hence, the capsule is considered an important virulence factor.
3. Some bacteria attach to the target surface by using their capsules or glycocalyx in order to establish infection. For instance, *Streptococcus mutans* form glycocalyx, with which the bacteria stick to the tooth enamel.



## Appendages

Several structures project through the cell wall of bacteria to form surface appendages. The most commonly observed are flagella and pili.

### A- Flagella

Many genera of bacteria move by means of flagella.

- Flagella are only 20 nm in diameter, too small to be detected by light microscope. They can be demonstrated clearly with the electron microscope.
- The location and number of flagella on a cell vary according to bacterial species. Organisms may be **monotrichous** (single polar flagellum), **lophotrichous** (multiple polar flagella) or **peritrichous** (flagella distributed over the entire cell surface) (Fig. 3).
- Flagella consist of a single type of protein called flagellin which differs in different bacterial species. The flagellins are highly antigenic (H antigen).

Motile bacteria tend to migrate towards regions where there is a higher concentration of nutrients and solutes (a process known as chemotaxis) and away from disinfecting substances (negative chemotaxis).

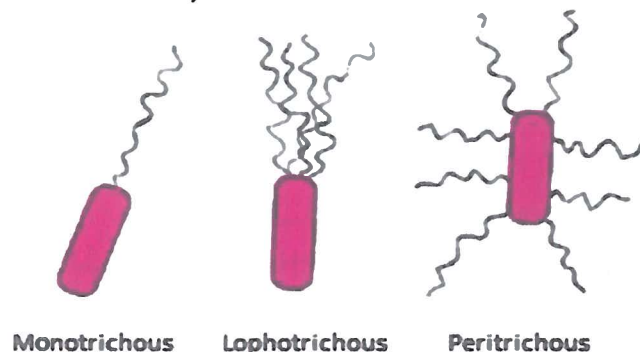


Fig. (3): Different distribution of flagella

### Axial filaments

These structures are composed of two groups of fibres that originate within the opposite ends of the cell and overlap in the middle. Structurally and chemically, the fibres of the axial filaments are similar to flagella and they are sometimes called "endoflagella". Spirochaetes move by means of these axial filaments. When the cell moves, it rotates around its longitudinal axis and flexes and bends along its length.

### B- Pili or fimbriae

Pili (singular: pilus) are protein tubes that extend from the cells. They are shorter and thinner than flagella and can be observed only by the electron microscope. They are composed of structural protein subunits termed pilins.

#### Functions:

1. **Adherence:** It is the function of the short pili (fimbriae) that occur in great numbers around the cell. They enable bacteria to attach to the surfaces, thus contributing to the establishment of infection i.e. virulence factor. For instance, *N. gonorrhoeae* withstands the flushing action of urine by adhering to the urethral mucosa.
2. **Conjugation:** A special long pilus called the sex pilus (F pilus) is involved in the transfer of DNA between bacteria, a process known as conjugation (Chapter 6).



## Bacterial Spores (Endospores) ?

Some bacteria, notably those of the genera *Bacillus* and *Clostridium*, develop a highly resistant resting phase or endospore that does not grow or reproduce, and exhibits absolute dormancy. A single vegetative bacterium forms a single spore by a process called **sporulation**. A single vegetative bacterium emerges from a spore during **germination**.

### Sporulation

Sporulation is triggered by the onset of unfavourable environmental conditions e.g. depletion of nutrients, accumulation of metabolites or changes in the growth requirements (e.g. moisture, temperature, pH, or oxygen tension).

The cytoplasmic membrane invaginates enclosing a section of the cytoplasm that contains the bacterial chromosome, some ribosomes and other cytoplasmic materials that will be needed for germination. It acquires a thick cortex and a thin but tough outer spore coat.

### Viability and resistance

Spores are much more resistant to disinfectants, drying and heating. Moist heat at 121°C for 10-20 minutes is needed to kill spores while 60°C suffices to kill vegetative forms. The marked resistance of the spores has been attributed to several factors:

1. Thermal resistance is provided by their high content of  $\text{Ca}^{2+}$  and dipicolinic acid (a compound unique to endospores).
2. The impermeability of their cortex and outer coat.
3. Their low content of water.
4. Their very low metabolic and enzymatic activity.

### Germination

Endospores respond quickly to environmental changes returning to the vegetative state within 15 min. In the process of germination, the spores absorb water and swell, the protective coat disintegrates and a single vegetative cell emerges.

### Morphology

1. **Staining:** Using Gram's stain, the spore remains uncoloured and can be seen as a clear area within the stained cell. The spores can be stained using special procedures.
2. **The position (Fig. 4):** In relation to the body of the bacillus, the spore may be central, terminal or subterminal.
3. **The shape:** The spores may be oval or rounded.

The position and shape of spores are characteristic of the species and may help in the microscopic identification of the bacterium.



Fig. (4): The position of spores

**MCQs:**

- 1- The following are functions of the cytoplasmic membrane **EXCEPT**:
  - a- Respiration
  - b- Cell wall biosynthesis
  - c- Reproduction
  - d- Staining affinity
  - e- Selective transport
- 2- Lipid A is a cell wall component of:
  - a- Gram positive bacteria
  - b- Gram negative bacteria
  - c- Fungi
  - d- Algae
  - e- Viruses
- 3- One of the following is a function of the cell wall:
  - a- Maintaining the characteristic shape of the bacterial cell
  - b- Selective transport
  - c- Respiration, since respiratory enzymes are located in it
  - d- Protein synthesis
  - e- Excretion of extracellular enzymes
- 4- All the following are characters of L-forms of bacteria **EXCEPT**:
  - a- They are naturally occurring bacteria without cell wall.
  - b- They are resistant to antibiotics which inhibit cell wall synthesis.
  - c- They develop only in isotonic media.
  - d- They can produce relapses of overt infections.
  - e- They may resynthesize the cell wall.
- 5- Bacteria are protected from phagocytosis by:
  - a- The capsule
  - b- Lipoprotein
  - c- The mesosome
  - d- The outer membrane
  - e- Peptidoglycan
- 6- All of the following are true concerning pili **EXCEPT**:
  - a- They mediate bacterial adherence.
  - b- They may be involved in bacterial conjugation.
  - c- Their antigen is called H antigen.
  - d- They are important virulence factors.
  - e- They are protein in nature.
- 7- The marked resistance of the spores can be attributed to all the following factors **EXCEPT**:
  - a- The impermeability of their cortex and outer coat
  - b- Their ability to resist phagocytosis
  - c- Their low content of water
  - d- Their very low metabolic and enzymatic activity
  - e- Their high content of Ca and dipicolinic acid



# BACTERIAL GROWTH AND PHYSIOLOGY

normal Functions → growth & replicat

## ILOs:

By the end of this chapter the student should be able to:

- Describe the process of binary fission النظار الثاني
- Define generation time
- Recall bacterial growth requirements nutrients < O<sub>2</sub>, CO<sub>2</sub>, PH, Temperature.
- Contrast events related to bacterial growth curve

Growth involves an increase in the size and number of organisms. In the laboratory, bacterial growth can be seen in one of two main forms: عنايتات النمو

1. Development of colonies, which are the macroscopic products of 20-30 cell divisions of a single bacterium on solid media. مستعمرات
2. Transformation of a clear fluid medium to a turbid suspension. number = 2<sup>30</sup> or 2<sup>20</sup>

## Bacterial Reproduction

Bacterial multiplication takes place by simple binary fission:

1. The cell grows in size, usually elongates. عالب
2. The bacterial chromosome acts as a template for the replication of another copy.
3. Each copy becomes attached to a mesosome on the cytoplasmic membrane.
4. The protoplasm becomes divided into two equal parts by the growth of a transverse septum from the cytoplasmic membrane and cell wall.

In some species, this septum splits the parent cell completely into two separate daughter cells. In others, the cell walls of the daughter cells remain continuous for some time after division giving the characteristic arrangement, e.g. pairs, clusters or chains. عنايت

**Generation time (doubling time):** is the time between two successive divisions. It may be as short as 13 min. in *Vibrio cholerae* and may reach 24 h. in *Mycobacterium tuberculosis*.

## Growth Requirements

In order to grow and divide, bacteria need the following growth requirements:

**1. Nutrients:** According to the means by which a particular organism obtains energy and raw material to sustain its growth, bacteria are classified into:

**a- Autotrophs:** They can utilize simple inorganic materials, e.g. CO<sub>2</sub> as a source of carbon and ammonium salts as a source of nitrogen. They can synthesize complex organic substances from the simple inorganic materials. The energy required for their metabolism is predominantly derived from light or simple chemical reactions. Autotrophs are of no or little medical importance.

الكروماتيلات النباتية حيازة عن كبريتا.

**b- Heterotrophs:** These bacteria, on the other hand, require organic sources for carbon, as they can not synthesize complex organic substances from simple inorganic sources. Most bacteria of medical importance are heterotrophic.

**2. Oxygen (O<sub>2</sub>):** According to O<sub>2</sub> requirements, bacteria are classified into:

- a- **Strict or obligate aerobes** require oxygen for growth, e.g. *Pseudomonas aeruginosa*.  
للزوم O<sub>2</sub>
- b- **Strict or obligate anaerobes** require complete absence of oxygen, e.g. *Bacteroides fragilis*.  
معتزها من الاكسجين الى هنتخلص منها - Wastes
- c- **Facultative anaerobes** generally grow better in presence of oxygen but still are able to grow in its absence, e.g. staphylococci, *E. coli*, ...etc.  
اختياري
- d- **Micro-aerophilic** organisms require reduced oxygen level, e.g. *Campylobacter* and *Helicobacter*.  
عندها كمية قليلة من الاكسجين
- e- **Aerotolerant anaerobes** have an anaerobic pattern of metabolism but can tolerate the presence of oxygen because they possess superoxide dismutase e.g. *Clostridium perfringens*.  
عندها الازيم واحد فيتركم هاتجدا له فياضروقتا اظلم على الخلية موت

• **Respiration and energy production:** The cellular respiration is another name of glucose catabolism. When it takes place in presence of oxygen, it is called aerobic cellular respiration. When it takes place in absence of oxygen, it is called anaerobic cellular respiration.

**Aerobic cellular respiration:** The glucose catabolism under aerobic conditions results in the production of energy in the form of 38 ATP molecules. The final electron acceptor is molecular O<sub>2</sub>. During this type of respiration, superoxide (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) are formed. These molecules are highly toxic. To cope with this, aerobic organisms have developed two enzymes, superoxide dismutase and catalase, which detoxify these molecules.

**Anaerobic cellular respiration:** It occurs in the absence of oxygen. The final electron acceptor is an inorganic molecule such as nitrate (NO<sub>3</sub><sup>-</sup>), sulfate (SO<sub>4</sub><sup>-2</sup>), or CO<sub>2</sub>. The net yield of ATP molecules is less than it is with aerobic cellular respiration because nitrate, sulfate, and CO<sub>2</sub> are not as good at accepting electrons as oxygen. Compared to aerobes, obligate anaerobes lack superoxide dismutase and catalase and so they can not grow in presence of O<sub>2</sub>.

**Fermentation:** It is an anaerobic process, because it takes place in the absence of oxygen. It is used by facultative anaerobes when they exist in an environment that does not contain a suitable inorganic final electron acceptor (NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>-2</sup> or CO<sub>2</sub>). This is the least efficient method of generating energy.

**3. Carbon dioxide (CO<sub>2</sub>):** The minute amount of CO<sub>2</sub> present in air is sufficient for most bacteria. However, certain species require higher concentrations (5-10%) of CO<sub>2</sub> for growth (capnophilic) e.g. *Neisseria spp.* and *Brucella abortus*.  
Capnophilic بتركيز CO<sub>2</sub> و لكن الكثير الى كتموال فها هو CO<sub>2</sub> Bacteria growth faster CO<sub>2</sub> لاجل

#### 4. Temperature:

- **Mesophiles** are organisms able to grow within a temperature range of 20-40°C. Pathogens which replicate on or in human body are able to grow within this range, with an optimum temperature of 37°C which is the normal body temperature.



- **Psychrophiles** (cold-loving) are capable of growth at refrigeration temperature (0-8°C), e.g. *Flavobacterium* spp. تبريد
- **Thermophiles** (heat-loving) grow best at high temperature (>60°C), e.g. *Bacillus stearothermophilus*. في الحرارة

**5. Hydrogen ion concentration (pH):** Most microorganisms of clinical significance grow best in media whose pH is close to that of human body (pH 7.2). However, some microorganisms grow better at an alkaline pH (8-9), such as *V. cholerae*. Others, such as lactobacilli, prefer media of acidic pH (4 or less).

### Growth Phases (Bacterial Growth Curve)

If a small number of an organism is placed in a suitable fluid nutrient medium under appropriate physical and chemical conditions, then the number of viable cells per millilitre is determined periodically, and plotted, a characteristic growth curve with four phases is obtained (Fig. 5): دور

- 1. Lag phase:** The initial number of bacterial cells remains constant. During this period, the cells adapt to their new environment. Enzymes and intermediates are formed to permit growth. التكيف
- 2. Exponential (logarithmic) phase:** There is marked increase in cell number and its rate is accelerated exponentially with time giving a characteristic linear plot on a logarithmic scale. In this phase, the organism shows typical morphology. تضاعف
- 3. Stationary phase:** Exhaustion of nutrients and accumulation of toxic products cause growth to decrease. There is slow loss of cells through death which is just balanced by formation of new cells through growth and division. The number of viable bacteria remains constant. generati. no waste product
- 4. Decline phase:** At the end of the stationary phase, the death rate increases and exceeds the multiplication rate due to nutrient exhaustion and accumulation of toxic metabolic end products. So, the number of viable bacteria decreases. موت سريع ويزيد قدام

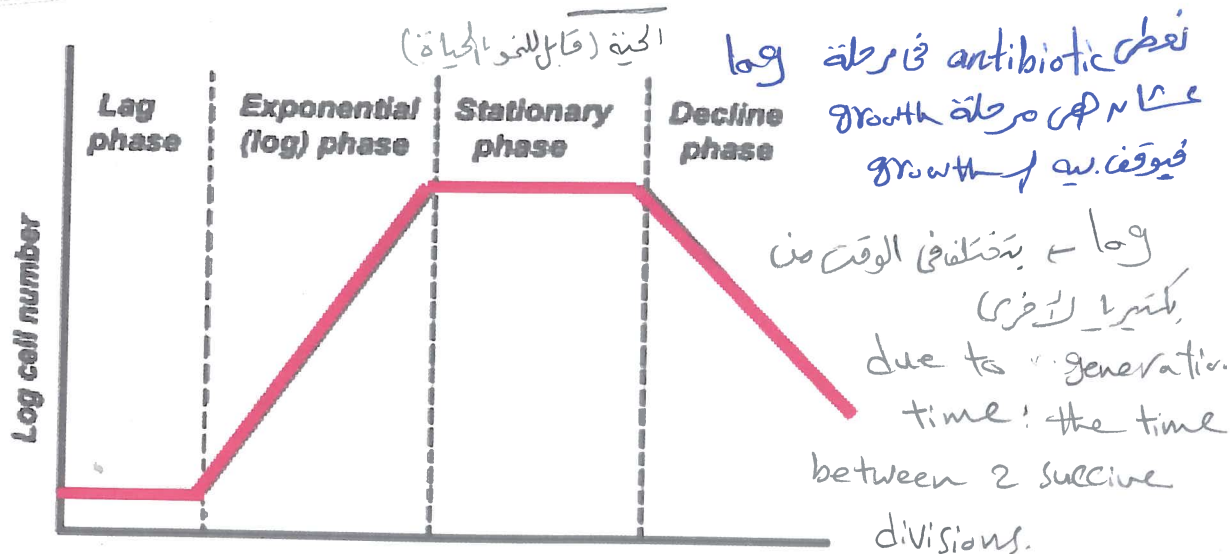


Fig. (5): Phases of bacterial growth curve

**MCQs:**

- 1- What types of bacteria synthesize organic compounds from inorganic compounds?  
a- Heterotrophs  
b- Obligate anaerobes  
c- Aerobes  
d- Facultative anaerobes  
☒ e- Autotrophs
- 2- Which of the following terms best describes bacteria that lack catalase but not superoxide dismutase?  
a- Obligate aerobe  
b- Obligate anaerobe  
c- Facultative anaerobe  
☒ d- Aerotolerant anaerobe  
e- Microaerophilic
- 3- Capnophilic bacteria require:  
a- Low concentration of  $O_2$   
b- High concentration of  $O_2$   
☒ c- High concentration of  $CO_2$   
d- Alkaline pH  
e- High temperature
- 4- What type of bacterium is most likely to cause spoilage of refrigerated foods?  
a- Mesophilic  
b- Thermophilic  
☒ c- Psychrophilic  
d- Capnophilic  
e- Microaerophilic
- 5- Bacterial cell death is balanced by the formation of new cells in:  
a- Lag phase  
b- Exponential phase  
☒ c- Stationary phase  
d- Decline phase  
e- Log phase

## BACTERIAL VIRUSES (BACTERIOPHAGES)

### ILOs:

By the end of this chapter the student should be able to:

- Recall structure of bacteriophage
- Contrast lytic and lysogenic replication cycles of bacteriophage
- Define lysogenic conversion
- Contrast generalized and specialized transduction
- List practical uses of bacteriophages

Bacteriophages (or phages) are viruses that parasitize bacteria i.e. the bacterial cell serves as a host for the virus.

### Morphology of the Bacteriophage: (Fig. 6)

In most cases, the bacteriophage consists of:

1. **A head:** containing the nucleic acid core (usually DNA, rarely RNA) surrounded by a protein coat (capsid).
2. **A tail:** consists of a hollow core surrounded by a contractile sheath which ends in a base plate to which tail fibres are attached.

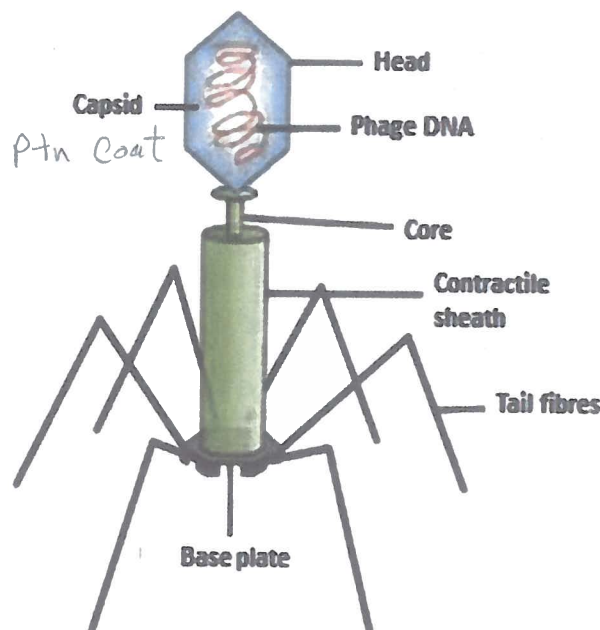


Fig. (6): Structure of a bacteriophage



## Replication (Propagation) of Bacteriophages

Two cycles for phage replication are known (Fig. 7):

### A- Lytic (vegetative) cycle:

It is so-called because it ends in lysis of the bacterial host cell and release of the newly formed phages. The stages of this cycle are:

- 1. Adsorption:** The phage attaches, by its tail, to specific receptors on the bacterial cell. The specificity of this process determines the susceptibility of bacteria to different phages.
- 2. Penetration:** The tail sheath contracts and the nucleic acid is injected into the cell. The empty head and the tail are left outside the cell.
- 3. Eclipse phase:** in which no phage components are detected inside the cell. It takes a short time (minutes to hours) during which viral nucleic acid directs the host cell metabolism to synthesize the enzymes and proteins required for phage synthesis.
- 4. Intracellular synthesis:** of phage nucleic acids, capsids and tails. Several hundreds of phage components are synthesized.
- 5. Assembly:** The phage components aggregate to form complete phage particles which mature into typical infectious phages.
- 6. Release:** The bacterial cell bursts liberating a large number of phage particles to infect new cells.

N.B.: During the lytic phage cycle, fragments of the bacterial DNA may be incorporated into the phage head. The phage can then transfer the incorporated bacterial DNA into another bacterial host "generalized transduction" (Chapter 6).

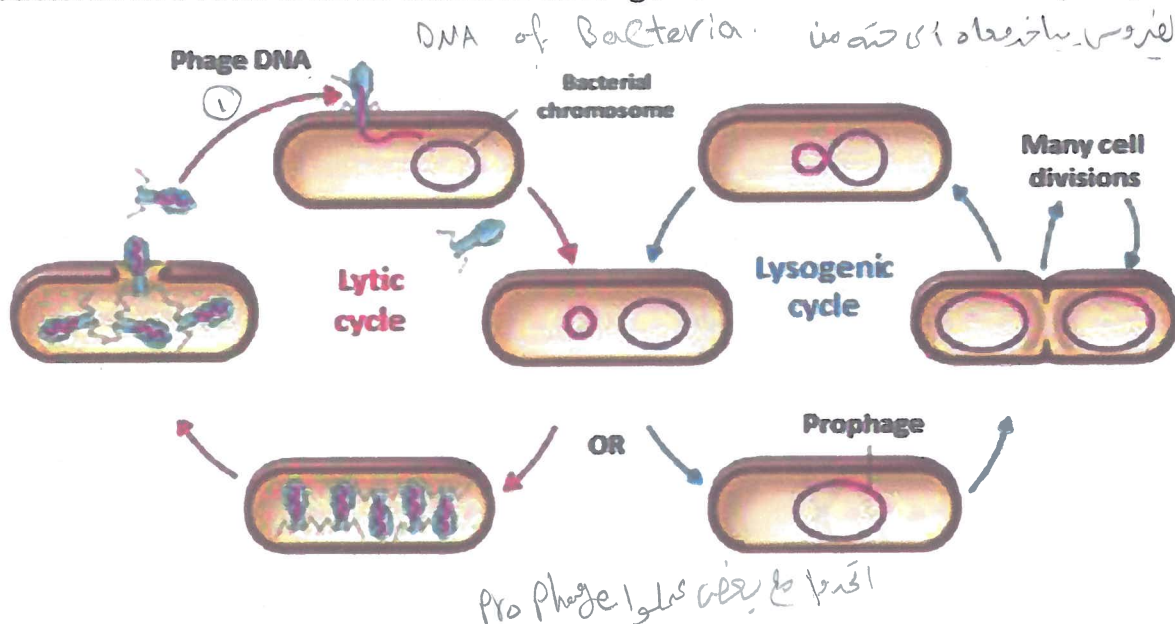


Fig. (7): The lytic and lysogenic cycles of bacteriophage

### B- Temperate (lysogenic) cycle

In this cycle, the phage (temperate phage) does not replicate and lyse the bacteria but the phage DNA becomes integrated with the bacterial chromosome and divides with it to pass into daughter cells. The integrated phage genome is called "prophage" and the bacteria carrying it are called "lysogenic" bacteria.



The presence of the prophage in the bacterium renders it:

1. Immune to infection by another phage.
2. Lysogenic: the bacterium acquires new properties, e.g. diphtheria bacilli can produce toxin only when lysogenized. Acquisition of a new character coded for by a prophage DNA is called "lysogenic conversion", or "phage conversion". When the phage is lost from the bacterium, this new characteristic is lost.

### Outcome of the temperate cycle

1. The prophage may be carried inside the bacterial cell indefinitely passing to daughter cells.
2. The prophage may be induced to detach from the bacterial chromosome and start a lytic cycle. Induction may be spontaneous or achieved by an inducer, e.g. U.V. light.

During the process of induction, the prophage may carry with it few genes of the bacterial chromosome. When it infects another bacterium, it passes this fragment to it giving it new characters. This is known as "specialized transduction" (Chapter 6).

### Practical Uses of Bacteriophages

1. Phages are used as cloning vectors in recombinant DNA technology. They carry and introduce foreign DNA fragments into a host cell.
2. Phage typing: Since bacteria differ in their sensitivity to different phages, phages are used to identify and type strains of bacteria that are biochemically and antigenically indistinguishable. This phage typing is important in epidemiologic studies, e.g. to trace the source of infection in outbreaks of post-operative wound sepsis caused by *Staphylococcus aureus*.
3. Phages are used as research elements in some biological and genetic studies.

### MCQs:

- 1- In lytic cycle of bacteriophages all the following occur **EXCEPT**:
  - a- Lysis of the bacterial host cell & release of newly formed phages.
  - b- The tail sheath contracts & nucleic acid is injected into the cell.
  - c- The phage attaches by its tail to a specific receptor.
  - ☒ d- The prophage is carried inside the bacterial cell indefinitely passing to daughter cells.
  - e- The phage components aggregate to form complete phage particles.
- 2- The lysogenic bacterial cell is the cell containing:
  - a- Lysosomes
  - b- Lysozymes
  - c- Bacteriocins
  - ☒ d- Prophage
  - e- Endospores

## BACTERIAL GENETICS

الوراثة

### ILOs:

By the end of this chapter the student should be able to:

- Define bacterial genome
- List components of bacterial genome Chromosome
- Describe bacterial chromosome Extra-Chromosome
- Describe plasmid structure
- List functions of plasmids
- Contrast relaxed and stringent plasmids
- Compare between conjugative and non-conjugative plasmids in relation to gene transfer
- Define transposable genetic elements
- Explain different classes of transposable genetic elements

علم الوراثة

**Genetics** is the science, which defines and analyzes heredity. The unit of heredity is the **gene**, a segment of DNA that carries information for a specific biochemical or physiologic property.

The bacterial **genome** is the total set of genes present inside the bacterial cell. It comprises:

1. The bacterial chromosome: that can encode up to 4000 separate genes necessary for bacterial growth and propagation.  
Additional genes may be carried on:
2. Plasmids,
3. Transposable genetic elements, and
4. Bacteriophage DNA (prophage).

extra  
Chromosome

Genotype:

Phenotype:

المورثة = C



## 1. The Bacterial Chromosome:

Being a prokaryote, the bacterial cell lacks a nuclear membrane; instead, the DNA is concentrated in the cytoplasm as a **nucleoid**. *نوية الخلية*

The nucleoid consists of a single chromosome, which is a circular, supercoiled, double-stranded DNA molecule, associated at one point with a mesosome. This attachment plays a role in the separation of the two sister chromosomes following chromosomal replication.

The bacterial chromosome has the general chemical structure of DNA molecules:

- Each strand is formed of regularly alternating phosphate and sugar (deoxyribose) groups.
- A nitrogenous base (A, G, C, or T) is attached to the sugar group and is projecting inwards towards the other strand.
- The two strands are held together by hydrogen bonds between complementary bases (A-T) or (C-G) present at the same level.
- The average length of the bacterial chromosome is 4000-5000 Kbp. *base pair*

The bacterial chromosome replicates by the semi-conservative method of DNA replication, i.e.: ?

- The two strands are separated.
- Each strand acts as a template to synthesize a complementary strand through the action of the polymerase enzyme.

The bacterial chromosome follows the same rules of gene expression and protein synthesis (i.e. transcription and translation) as higher organisms.

## 2. Plasmids

Plasmids are extra-chromosomal, circular, double-stranded DNA molecules dispersed in the cytoplasm. They are much smaller than the bacterial chromosome (from several to 100 Kbp). *لوسيتا في 2.5 كبر*

Plasmids are capable of replicating independently of the bacterial chromosome. Thus, multiple copies of the same plasmid may exist in the same cell (**plasmid copy number**). According to the copy number, plasmids can be categorized into 2 groups: *8/3*

1. **Relaxed replicating plasmids:** They can replicate in the absence of protein synthesis. They are usually present in 30-50 copies/cell, and are relatively small in size.
2. **Stringent plasmids:** They require protein synthesis, and are usually large and present in a few copies (1-5) per cell. *غير ضروري*

Plasmids are generally dispensable. This indicates that most plasmids encode properties that are not essential for growth, replication or survival of the host bacterium. This is evidenced by: *بقاء*

- a- The spontaneous loss of plasmids during cell division.
- b- Plasmid curing: The experimental kicking off of the plasmids using physical agents (e.g. heat) or chemical agents (e.g. antibiotics). *يترك البلازميد يهرب*

*علاج = Curing*



### Functions (traits) exhibited by plasmids

1. **Sex pilus formation:** Some plasmids carry fertility (F) factors that code for the formation of a sex pilus which mediates the process of conjugation. For this reason, such plasmids are also known as conjugative plasmids (Table 1).

Table (1): Comparison between conjugative and non-conjugative plasmids

	Conjugative plasmids	Non-conjugative plasmids
Size	Large	Usually small
Copy number	1-2 (stringent)	>30 (relaxed)
F factors	Present	Absent
Sex pilus formation	Yes	No
Transfer among Bacteria	by conjugation الأقران	by the help of a conjugative plasmid
Host bacteria	Common in Gram -ve bacilli	Common in Gram +ve cocci

2. **Antibiotic resistance:** Some plasmids carry genes for resistance (R-factors) to one or several antimicrobial drugs. They often control the formation of enzymes capable of destroying the antimicrobial drugs, e.g.  $\beta$ -lactamase enzyme which determines resistance to penicillin and cephalosporins.

R-factors are usually conjugative plasmids that can be transferred among bacteria by conjugation. This results in the rapid spread of drug-resistance among bacterial populations and the development of multiple drug-resistant bacterial strains.

3. **Virulence plasmids:** may code for exotoxins, adhesins or invasion factors.

مواد بروتينية تولدونها بهذا البكتيريا لتعطيها أنواعاً أخرى

4. **Bacteriocin production:** Bacteriocins are bactericidal substances produced by certain bacterial strains and are active against other strains of the same or closely related species, e.g. colicin E1 produced by *E.coli*.

5. **Other functions** include nitrogen fixation, sugar fermentation, antibiotic production,  $H_2S$  production, resistance to heavy metals and degradation of aromatic compounds.

### 3. Transposable Genetic Elements

These are non-replicating DNA segments that are capable of inserting themselves into other DNA molecules. They are also capable of mediating their own transfer from one location to another on the same chromosome or between chromosomes and plasmids.

Transposition occurs infrequently (once every  $10^5$ - $10^7$  generations) often in a random pattern.

The insertion of a transposable element into a gene usually leads to inactivation of that gene.

السلوك العشوائي

There are different classes of transposable genetic elements, for example:

- Transposons, which encode specific genes (such as antibiotic resistance)
- Pathogenicity islands (PAI), which give the bacterium a variety of virulence characters, such as the ability to adhere to or invade host cells.

#### 4. Bacteriophage DNA

*Bacteriophage* فيروس آكل للبكتيريا

The DNA of the temperate bacteriophage that is integrated in the chromosome of a lysogenic bacterial cell (i.e. the prophage) is considered as a part of the genome of such bacteria (see chapter 4).

#### MCQs:

- Bacterial genetic information is carried on the following EXCEPT:**
  - ☒ Ribosomes
  - ☐ Chromosome ✓
  - ☐ Transposons
  - ☐ Plasmids ✓
  - ☐ Bacteriophage ✓
- Plasmids:**
  - Are single-stranded DNA molecules *double*
  - ☒ Carry optional genes (are dispensable) ✓
  - ☐ Carry genes essential for growth ✗
  - Are always found in linear form *circular*
  - Are always present as one copy/cell *30-50 copies/cell*
- Plasmids differ from transposable genetic elements, as plasmids:**
  - Become inserted into chromosomes ✗
  - Are self-replicating outside the chromosome ✓
  - Move from chromosome to chromosome ✗
  - Carry genes for virulence (e.g. exotoxin production) ✓
  - Carry genes for antibiotic resistance
- Plasmids may code for any of the following EXCEPT:**
  - Sex pilus formation
  - Bacteriocin production
  - ☒ Growth
  - Antibiotic resistance
  - Virulence
- Conjugative plasmids:**
  - Are usually small in size *large*
  - ☒ Carry fertility (F) factor
  - Are relaxed plasmids ✗
  - Have a large copy number ✗
  - Are common in Gram positive cocci

BACTERIAL VARIATION

ILOs:

By the end of this chapter the student should be able to:

- Define bacterial variation
- Contrast phenotypic and genotypic variation
- Define mutation
- List types of mutation
- Describe methods of gene transfer

Bacterial variations are changes in the bacterial characters. They may be phenotypic or genotypic (Table 2).

Table (2): Comparison between phenotypic and genotypic variations:

Phenotypic variation	Genotypic variation
- It occurs in response to changes in the environmental conditions without change in the genetic constitution	- It occurs as a result of a change in the underlying genetic constitution
- Reversible (transient)	- Irreversible (permanent)
- Not-heritable	- Heritable
- Examples: 1. L-forms of bacteria 2. Loss of flagella upon exposure to phenol	- It occurs through: 1. Mutation 2. Gene transfer: a- Transformation b- Transduction c- Conjugation



## Mutation

It results from a change in the nucleotide sequence of DNA that may occur **spontaneously** as a replication error (at a rate of once every  $10^6$ - $10^7$  cells), or may be **induced** by radiation or chemical agents (at a higher rate of once every  $10^3$ - $10^4$  cells).

Mutation can be classified according to nucleotide substitution, insertion or deletion into:

1. **Single-base (point) mutations:** involve the replacement (substitution) of a single nucleotide in the coding sequence. This may result in:
  - a- Same sense (silent) mutations: occur when the resulting base triplet (codon) codes for the same amino acid as the original triplet.
  - b- Missense mutations: occur when the mutant base changes the coding sequence so that a different amino acid is produced. The resulting protein may be functioning or not, depending on the importance of the area affected by the mutation.
2. **Frame-shift mutations:** occur when a nucleotide is inserted into, or deleted from the coding sequence, resulting in a shift of the reading frame, e.g. insertion of a transposable element.

Induced mutations may be used to manipulate viral genomes for vaccine production and gene therapy.

## Gene Transfer

There are 3 methods for gene transfer among bacteria (Fig. 8):

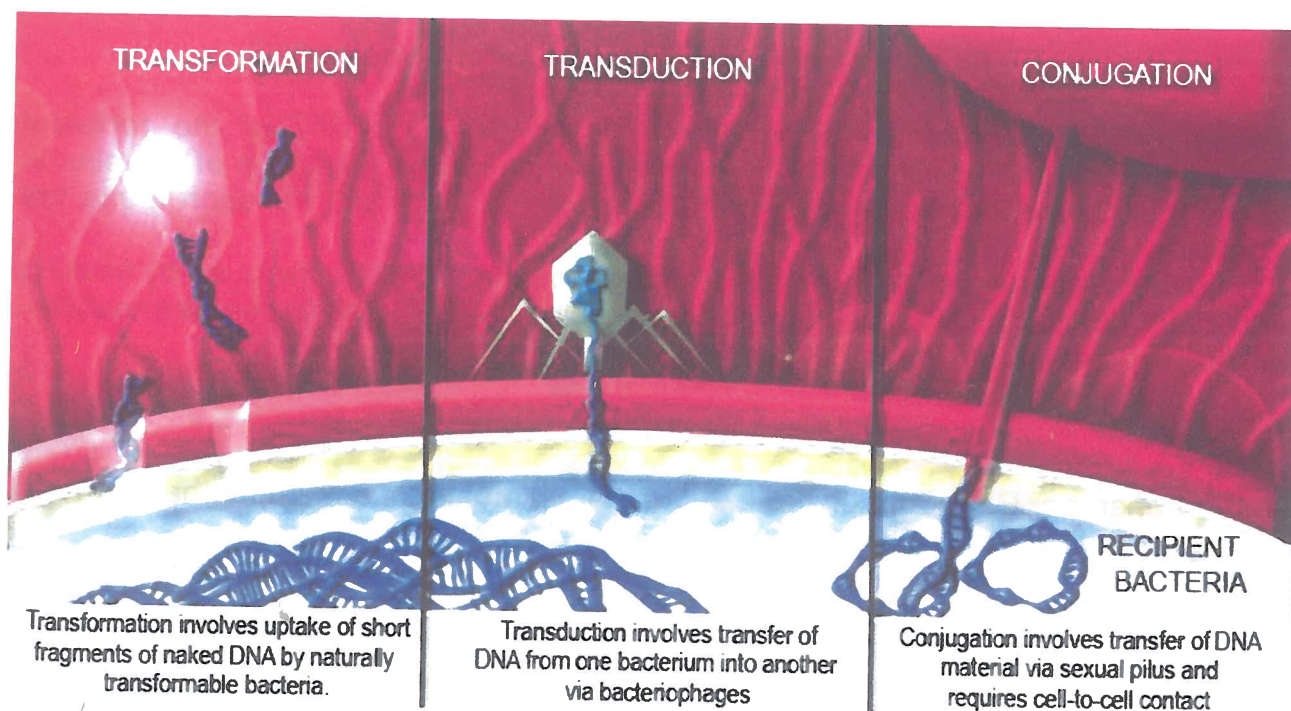


Fig. (8): Methods of gene transfer

### 1. Transformation: (Fig. 9)

Dying bacteria release DNA which can be taken up by other bacteria. Such DNA may be chromosomal or plasmid in origin, and may carry genes that "transform" the recipient bacterium.

The transforming DNA may become integrated with the bacterial chromosome or re-established extrachromosomally in the recipient cell.

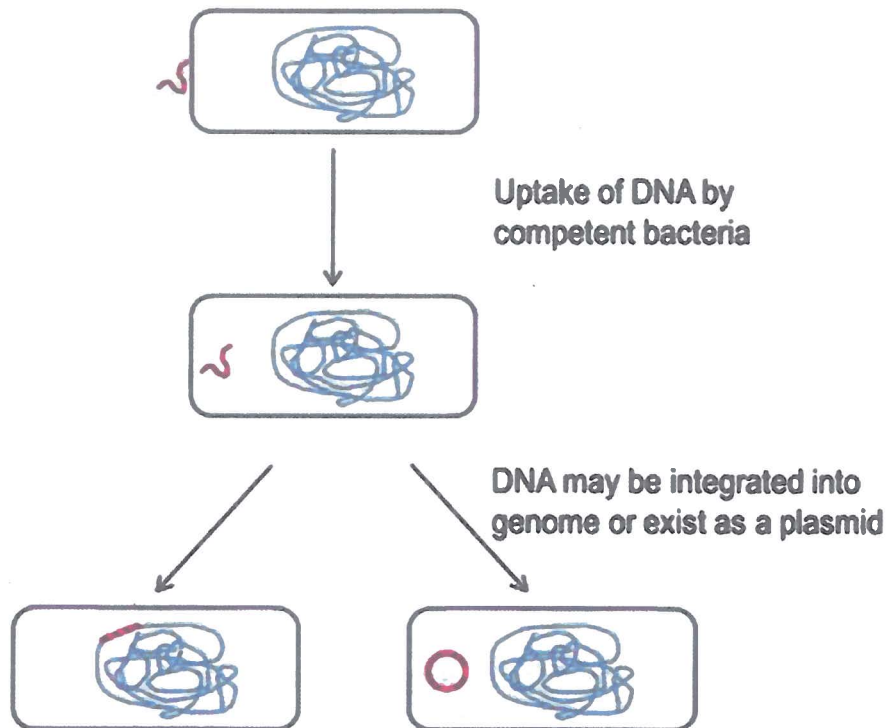


Fig. (9): Gene transfer by transformation

Transformation depends on **competence**, which is the ability of the recipient bacterial cell to take up DNA. Competence depends on the presence of proteins in the cell membrane that have a special affinity to bind DNA and transport it into the cytoplasm.

Artificial competence can be induced during recombinant DNA techniques by treating the recipient bacteria with **calcium chloride**, which alters cell membrane permeability, enabling the uptake of DNA.

### 2. Transduction

It is the transfer of DNA from one cell to another by means of a bacteriophage. There are 2 types of transduction:

#### a- Generalized transduction:

During the lytic phage cycle, the bacterial DNA is fragmented and any fragment of DNA (whether chromosomal or plasmid) may be incorporated into the phage head. The phage particle can then transfer the incorporated bacterial DNA into another bacterial host.

في Lytic Cycle البكتريا يقوت وال Phage ياكل DNA بتاعها ياكله في حته من generalized  
 في 11 lysogenic لا يقوت ال prophage ياكله معاه حته اللى هو كان ساكن فيها .



**b- Specialized transduction:**

It takes place when a prophage contained in a lysogenized bacterial cell is induced to detach. Such prophage may carry with it the adjacent piece of the chromosomal DNA and transfer it to another bacterial cell.

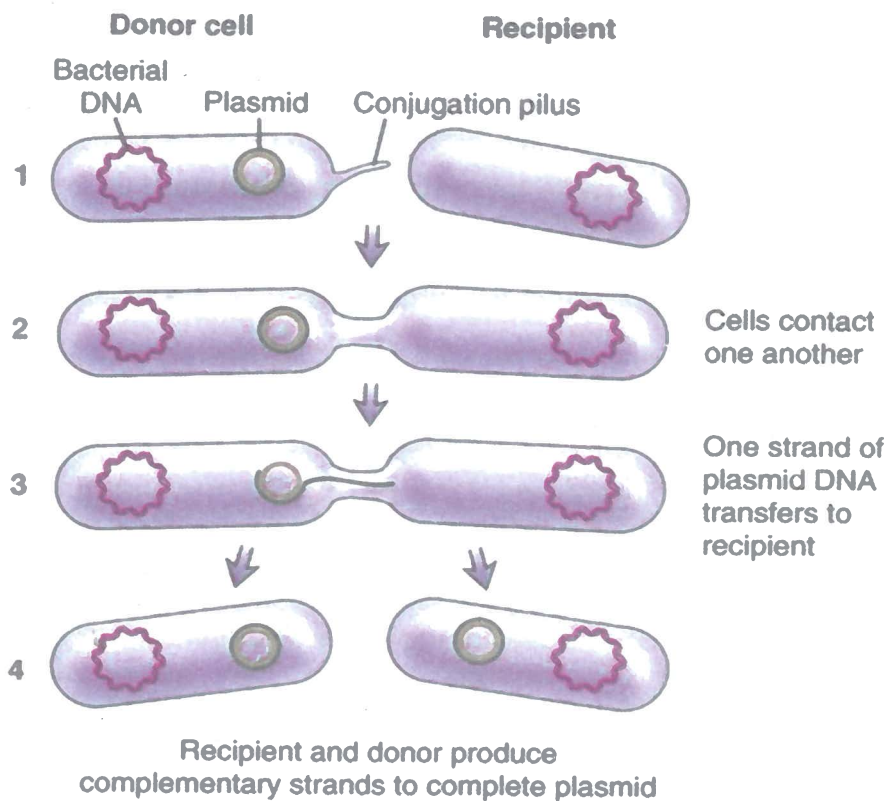
**Table (3):** Comparison between generalized and specialized transduction:

	Generalized transduction	Specialized transduction
Type of phage	Lytic (virulent) phage	Temperate (lysogenic) phage
Replication cycle	Lytic cycle	Lysogenic cycle
The transferred DNA fragments	Any piece of bacterial DNA (chromosomal or plasmid)	A specific piece of chromosomal DNA adjacent to the site of insertion of the prophage

**3. Conjugation:** (Fig. 10)

It is the most frequently observed mechanism of DNA transfer. It involves 2 cell types: donors ( $F^+$ ) which possess the fertility (F) factor, and recipients ( $F^-$ ) which lack the F factor.

The F factor carries the genes for the synthesis of the sex pilus which acts as a conjugation tube between the donor and recipient bacterial cells. The 2 DNA strands of the F factor are then separated, and one strand is transferred from the donor to the recipient cell. Each strand forms a complementary strand, thus, the recipient cell acquires a copy of the F plasmid and becomes an  $F^+$  cell

**Fig. (10):** Gene transfer by conjugation



**MCQs:**

- 1- Transformation in bacteria depends on:
  - a- F factors
  - b- R factors
  - c- Bacteriophages
  - d- Cosmids
  - e- Competence of bacteria
- 2- One of the following requires cell to cell contact:
  - a- Transformation
  - ☒ b- Conjugation
  - c- Transduction
  - d- Transcription
  - e- Transposition
- 3- Which of the following is mediated by a bacteriophage that carries host cell DNA:
  - a- Transformation
  - b- Conjugation
  - ☒ c- Transduction
  - d- Translation
  - e- Transcription
- 4- Regarding generalized transduction:
  - ☒ a- It occurs during lytic cycle of bacteriophage.
  - b- It occurs during lysogenic cycle of prophage.
  - c- A specific piece of bacterial DNA is transferred from one cell to another.
  - d- Sex pilus is necessary.
  - e- It results in phenotypic variation of bacterial character.

genotypic → gene transfer → transduction → phenotypic

## ANTIMICROBIAL CHEMOTHERAPY

### ILOs:

By the end of this chapter the student should be able to:

- Define the term antimicrobial chemotherapeutic agent
- Define the term antibiotic
- Define the term selective toxicity
- Recognize spectrum of activity of different antibiotics
- Describe different mechanisms of action of antibiotics with examples
- Explain principles of choice of antimicrobial agents
- Define the terms MIC and breakpoint
- List methods of antimicrobial susceptibility testing
- Define the term empiric therapy
- List indications of empiric therapy
- Recognize when to use combined therapy
- Explain outcomes of combined therapy
- Recall possible complications of antimicrobial agents with examples
- Recognize origin of resistance to antimicrobial agents
- List mechanisms of resistance to antimicrobial agents
- Define the term chemoprophylaxis
- Explain situations that require antimicrobial chemoprophylaxis

Bacter  
viral  
Fungi

**Antimicrobial chemotherapeutic agents:** are chemically synthesized substances that are used to treat infectious diseases by killing or inhibiting the growth (or multiplication) of microorganisms.

**Antibiotics:** are low-molecular weight antimicrobial substances that are produced as secondary metabolites by certain groups of microorganisms, especially *Streptomyces*, *Bacillus*, and a few moulds (*Penicillium* and *Cephalosporium*).

Although their original source was a microorganism, some antibiotics are currently made synthetically (**synthetic antibiotics**). Chemical modification of certain antibiotics, to achieve the desired properties, has been a prominent method of new drug development (**semisynthetic antibiotics**).

**Bacteriostatic agent:** is an antimicrobial agent that is capable of inhibiting bacterial multiplication. Multiplication resumes upon removal of the agent.

**Bactericidal agent:** is an antimicrobial agent that is capable of killing bacteria. Multiplication can not be resumed.

**Selective toxicity:** is the ability of an antimicrobial agent to harm a pathogen without harming the host. It may be a function of a specific receptor (or target) for the drug found in the microbe but not in the human body (e.g. peptidoglycan), or it may depend on the inhibition of a biochemical event essential for the organism but not for the host.

**Spectrum of activity:** the range of microorganisms that are affected by a certain antibiotic is expressed as its **spectrum of action**. Antibiotics which kill or inhibit the growth of a wide range of Gram-positive and Gram-negative bacteria are said to be **broad spectrum**. If effective mainly against either Gram-positive or Gram-negative bacteria, they are **narrow spectrum**. If effective against a single organism or disease, they are referred to as **limited spectrum**.

## Mechanisms of Action of Antimicrobial Agents

### A- Inhibition of bacterial cell wall synthesis

Agents acting by this mechanism include:

1.  $\beta$ -lactam antibiotics: e.g. penicillins, cephalosporins, and others.
2. Glycopeptides: e.g. vancomycin, and teicoplanin.
3. Cycloserine and bacitracin.

These antibiotics are bactericidal with minimal tissue toxicity.

The  $\beta$ -lactam drugs inhibit the last steps of peptidoglycan synthesis. This inhibition is initiated by binding of the drug to certain cell receptors known as penicillin-binding proteins (PBPs).

On the other hand, glycopeptides and cycloserine inhibit early steps in the biosynthesis of peptidoglycan, which occur inside the cytoplasmic membrane. Therefore, the mechanism of resistance to  $\beta$ -lactam antibiotics is different from that for the other groups. Subsequently, vancomycin could be used successfully in infections caused by  $\beta$ -lactam resistant staphylococci.

Q: why? vancomycin can kill penicillin resistant bacteria.

### B- Interference with the cell membrane function

Some agents disrupt the cytoplasmic membrane and interfere with its function. These include:

1. Antibacterial agents: e.g. polymyxin and colistin.
2. Antifungal agents: e.g. amphotericin B, nystatin and imidazoles.

These agents are microbicidal. They are highly toxic as they have narrow margin of selective toxicity.



### C- Inhibition of bacterial protein synthesis

Bacteria have 70S ribosomes (with 30S and 50S subunits) whereas mammalian cells have 80S ribosomes (40S and 60S subunits). This difference makes bacterial ribosomes a selective target for antimicrobials: *Explain why?* *لأنه يميز*

1. Agents acting on the 30S ribosomal subunit: e.g. tetracycline and aminoglycosides (gentamicin, amikacin, streptomycin).
2. Agents acting on the 50S ribosomal subunit: e.g. macrolides (erythromycin, azithromycin), lincomycins (clindamycin), streptogramins, linezolid, chloramphenicol and fusidic acid.

### D- Inhibition of bacterial nucleic acid synthesis

This may occur by:

1. Inhibition of RNA synthesis through the strong binding to DNA-dependent RNA polymerase: e.g. rifampin.
2. Inhibition of DNA synthesis through blocking DNA gyrase: e.g. quinolones and novobiocin.
3. Inhibition of dihydrofolic acid reductase leading to inhibition of folic acid synthesis. The latter is important for purine synthesis and, consequently, nucleic acid formation. Examples of these antimicrobials include trimethoprim and pyrimethamine.
4. Inhibition of folic acid synthesis by competitive antagonism e.g. sulphonamides. For many organisms, para-amino benzoic acid (PABA) is essential for the synthesis of folic acid. Sulphonamides are structural analogues of PABA. They compete with PABA for the active centre of the enzyme involved in folic acid synthesis. As a result, nonfunctional analogues of folic acid are formed and nucleic acid synthesis is inhibited.

## Choice of an Antimicrobial Agent for Therapy

The following are guidelines that can be followed for proper antibiotic use:

1. Select an antibiotic that is able to penetrate to the site of infection and achieve effective concentration, e.g. certain drugs are able to pass the blood-brain barrier, others are highly concentrated in urine.
2. Identify the nature of the infection whether bacterial, viral, fungal, or parasitic. A common mistake is to give an antibacterial agent for a viral infection.
3. Choose as narrow an antibiotic spectrum as you can. When you get the results of culture and susceptibility, *revise* your treatment to 'narrow-down' the spectrum as far as possible. The use of broad spectrum antibiotics is likely to faster induce resistance to antibiotics and may be complicated by superinfection. *لأنه يميز* *بroad spectrum*
4. Give the appropriate dose of the antibiotic for the proper duration. Inadequate dosage or undue prolonged therapy may result in drug toxicity and antibiotic resistance. *بوت - را - فتنه* *Pathogen*
5. Know the potential of the drug to produce toxicity: Some drugs known to be of low toxicity will exert high toxicity if they accumulate in the blood due to liver or kidney dysfunction. Use antibiotics that are only safe for the pregnant and lactating women and for infants and children.
6. Choose bactericidal rather than bacteriostatic antibiotics.

## Microbial Susceptibilities to Antimicrobial Agents

Microorganisms vary in their susceptibility to different chemotherapeutic agents, and susceptibilities can change over time. Ideally, the appropriate antibiotic to treat any particular infection should be determined *in vitro* before any antibiotic is given.

The *in vivo* activity of an antimicrobial agent is not always the same as its *in vitro* susceptibility because it involves many host factors that are not tested *in vitro*.

The activity of an antimicrobial agent against an organism is dependent on its concentration. Some idea of the effectiveness of a chemotherapeutic agent can be obtained from determining the **minimal inhibitory concentration (MIC)**. The MIC is defined as the lowest concentration of a drug that prevents growth of a test organism. The MIC forms the basis for susceptibility and determining breakpoints.

The **breakpoint** of an antimicrobial agent is the concentration that can be achieved in the serum with optimal dose. Organisms with MICs at or below the breakpoint are considered **susceptible**. On the other hand, organisms with MICs above the breakpoint are considered **resistant**.

Routine *in vitro* susceptibility testing can be done by one of the following methods:

1. Disc diffusion method.
2. Dilution method such as tube broth dilution.
3. Gradient diffusion (E test) method.

## Empiric Therapy

The empiric therapy is a "best guess" procedure based upon a provisional diagnosis made by the physician that a patient has a bacterial infection which requires treatment. Depending on the type of infection, there will be a short list of bacteria most likely to be causing that infection. Depending on the type of bacteria, there will be an antibiotic most likely to successfully treat that infection. "Best guess" treatment is not always successful or appropriate as many bacteria have unpredictable susceptibilities to antimicrobial agents.

### Indications:

1. In seriously ill patients **empiric therapy** should be started without delay but after collecting specimens for culture.
2. In closed lesions, where there is no available sample.

## Combined Therapy

The ideal rule in antimicrobial therapy is **mono-therapy** which means choosing **one** drug effective against a particular organism. However, there are conditions which necessitate the use of more than one antibiotic in order to achieve a successful clinical response.



### Possible indications

1. Severely ill patients suspected of having serious infections, e.g.:
  - Bacterial meningitis.
  - Sepsis in immunocompromised patients caused by *Pseudomonas aeruginosa*, *Klebsiella* spp., *Enterobacter* spp. or *S. aureus*.  
*in blood*
2. Febrile neutropaenia. *neutrophils* نقص
3. To delay the emergence of drug-resistant mutants e.g. in treatment of T.B.
4. To achieve bactericidal action through synergistic effect e.g. in enterococcal endocarditis. *ساحلي*
5. Mixed infections e.g. infections following massive trauma.

### Effects of combined therapy

1. **Synergistic effect ( $1+1 \neq 2$ ):** The combined action is significantly greater than the sum of both effects, e.g.:

- Vancomycin + gentamicin in treatment of methicillin-resistant staphylococci.
- Sulfamethoxazole + trimethoprim (cotrimoxazole) in treatment of shigellosis.

2. **Antagonistic effect ( $1+1 \neq 1$ ):** The combined action is less than that of the more effective agent when used alone, e.g.:  
Penicillin + chloramphenicol in treatment of meningitis. *Shigella Bal* *دواء* *effect* *أقل من الأكبر*

3. **Indifference ( $1+1 = 1$ ):** The combined action is no greater than that of the more effective agent when used alone, e.g.:  
Cefepime + vancomycin, or clindamycin + vancomycin. *مضادتين مع بعضهما*

4. **Addition ( $1+1 = 2$ ):** The combined action is equivalent to the sum of the actions of each drug when used alone. *جمع*

### Complications of Chemotherapy

1. **Toxicity:** may be dose-dependent or independent, e.g.:

- Tetracycline may cause staining of teeth in infants.
- Streptomycin may affect the 8<sup>th</sup> cranial nerve leading to vestibular dysfunction. *التأثير* *vestibulo*
- Aminoglycosides may cause nephrotoxicity.
- Chloramphenicol can cause bone marrow depression.

2. **Allergy (hypersensitivity):** usually not dose-dependent, e.g.:

- Penicillins may cause urticaria, anaphylactic shock or serum sickness.
- Local application of sulphonamides may result in contact dermatitis.

3. **Emergence of resistant strains:** The abuse of antibiotics (low dosage, interrupted course, no real indication, and improper choice) encourages the emergence of resistant mutants. These mutants will overgrow and replace the originally susceptible bacteria. It is recommended that *in vitro* susceptibility testing should be performed to guide the selection of antibacterial drugs. *أسوأ استعمال*

4. **Superinfection:** It occurs as a result of outgrowth of resistant members of normal flora when the sensitive ones are eradicated during antibiotic therapy e.g.:

- Pseudomembranous colitis caused by outgrowth of *Clostridium difficile*. *تحويل*
- Oral thrush caused by overgrowth of the yeast *Candida*. *normal flora into pathogenic*



## Resistance to Antimicrobial Agents

Antibiotic resistance is a global problem faced today in the treatment of infectious diseases. Resistance to antibiotics is more prevalent in hospitals especially intensive care units due to the higher antibiotic use. Resistance to antimicrobial agents is of two categories either intrinsic or acquired.

### Intrinsic (inherent or natural) resistance

This type of resistance refers to bacteria that are insensitive, in their natural state, to an antibiotic without the acquisition of resistance factors. It is consistent and can be expected once the organism is known. Intrinsic resistance occurs in the following situations:

1. *Streptomyces* are protected from the antibiotics they produce.
2. Gram-negative cell membrane has pores too small to allow large antibiotic molecules, e.g. nafcillin, to penetrate.
3. An organism lacks the target or receptor for the antibiotic as in the case of resistance of *Enterococcus* species to cephalosporins.

### Acquired resistance

It results from altered bacterial physiology and structure due to changes in the genome of the organism. It is inconsistent and unpredictable. The unpredictable nature of this resistance is the 1<sup>st</sup> reason why laboratory methods to detect resistance are necessary.

Acquired resistance mechanisms are driven by two **genetic processes** in bacteria:

(1) **Mutation and selection** (sometimes referred to as vertical evolution): Exposure of an organism to an antibiotic exerts a selective pressure on the organism and leads to mutation. The more frequent the exposure to the antibiotic the greater the potential resistance.

(2) **Exchange of genes** between strains and species (sometimes called horizontal evolution). Resistance genes can be encoded on plasmids, phages and transposable genetic elements (see chapter 5).

### Mechanisms of acquired resistance

Bacteria have the ability to use one or more of the following mechanisms:

#### A) Reduction of the intracellular concentration of the antibiotic by:

##### a. Decrease in influx of antibiotic through:

- Reduction of permeability of the outer membrane by modification or
- Loss of porin (a hollow membrane protein) required for entry of the antibiotic molecules.

b. **Efflux pumps**: The antibiotic is pumped out across the cytoplasmic membrane faster than it can diffuse in, so the concentration of antibiotic remains too low to be effective.

#### B) Inactivation of the antibiotic, e.g.:

- Production of  $\beta$ -lactamases leads to hydrolysis of the  $\beta$ -lactam ring, thus inactivating penicillins and cephalosporins.
- Production of acetyl transferase results in chloramphenicol resistance.
- Production of aminoglycosides-modifying enzymes.

- C) Target modification:** Modification of the target site for the antibiotic results in a reduced affinity for its receptor:
- Modification of the penicillin-binding proteins (PBPs) is a primary mode of resistance to  $\beta$ -lactam antibiotics in methicillin-resistant *S. aureus* (MRSA).
  - Alteration of the 50S ribosomal subunit reduces the affinity of macrolides linezolid and streptogramins for the ribosome.
  - Alteration of the 30S ribosomal subunit reduces the affinity of aminoglycosides for the ribosome.
- D) Target elimination by developing new metabolic pathways:** These bacteria have the ability to create new metabolic pathways that bypass the original target, e.g. resistance to trimethoprim.
- E) Target overproduction:** This may be the mechanism used by *S. aureus* strains with intermediate susceptibility to vancomycin (VISA).

## Antimicrobial Chemoprophylaxis

Chemoprophylaxis is the administration of an effective antimicrobial agent to prevent rather than to treat infection with a certain microbe, thus preventing development of a disease. Examples:

- **Long acting penicillin (or erythromycin):** is given to rheumatic patients to prevent reinfection with *S. pyogenes*.
- **Rifampicin:** is given to close contacts of meningococcal meningitis for 2 days to prevent meningitis.
- **Penicillin or erythromycin:** is given to individuals with abnormal heart valves prior to dental procedures to prevent endocarditis.
- **Preoperative** in some surgical operations.

### MCQs:

- 1- **Selective toxicity of an antibiotic:**
- Depends on presence of a receptor for the drug in hosts not in organisms
  - Is the ability of the drug to inhibit growth of a wide range of bacteria
  - Depends on inhibition of a biochemical event essential for the host
  - ☒ Is the ability of the drug to harm the organism without harming the host
  - Is one of the complications of antibiotic therapy
- 2- **Which of the following antimicrobial agents is most toxic to humans?**
- Bacitracin x
  - Cephalosporin x
  - ☒ Amphotericin B
  - Penicillin x
  - Vancomycin x

Cell wall  
Cell membrane  
host

amphotericin B  
هو ده مشترك مع البكتيريا و  
الخلايا



- 3- One of the following antimicrobial drugs is not among the group acting through inhibition of the bacterial cell wall:
- a- Penicillin ✓
  - b- Vancomycin ✓
  - c- Cephalosporins ✓
  - d- Bacitracin ✓
  - ☒ e- Novobiocin
- 4- Which of the following antibiotics inhibits bacterial protein synthesis by acting on the 30S ribosomal subunit?
- a- Vancomycin ×
  - b- Macrolides S.S.
  - c- Polymyxin ✓ mem
  - ☒ d- Aminoglycosides
  - e- Chloramphenicol × S.S.
- 5- The following are mechanisms of acquired resistance to antimicrobial agents EXCEPT:
- a- Decreasing the influx of the antibiotic
  - b- Modification of the receptor (target) site
  - c- Target elimination by developing new metabolic pathways
  - d- Target overproduction
  - ☒ e- Absence of cell wall
- 6- The MIC is the:
- a- Highest concentration of a drug required to inhibit bacterial growth
  - b- Standard dose of a drug required to inhibit bacterial growth
  - ☒ c- Lowest concentration of a drug required to inhibit bacterial growth
  - d- Lowest dilution of the a drug required to inhibit bacterial growth
  - e- Maximum dose of a drug required to inhibit bacterial growth
- 7- Combined antibiotic therapy is indicated in the following conditions EXCEPT:
- a- Mixed infections
  - b- T.B.
  - ☒ c- Viral meningitis
  - d- Endocarditis
  - e- Febrile neutropenia
- 8- Regarding the effect of combined therapy with antimicrobial drugs, the expression, " $1 + 1 = >2$ " means:
- a- Antagonistic effect
  - ☒ b- Synergistic effect
  - c- Indifference
  - d- Addition
  - e- Ineffectiveness



## DISINFECTION AND STERILIZATION

### ILOs:

By the end of this chapter the student should be able to:

- Define terms: sterilization, disinfection, disinfectant, antiseptics, germicide and sterilant
- Recognize levels of disinfectants with examples
- Define the term precleaning
- Define the term decontamination
- Describe main methods of disinfection
- Describe main methods of sterilization
- Compare between moist heat and dry heat in sterilization

### Definition and principles of terms

#### • Sterilization:

Validated process used to render a product free of all forms of viable microorganisms including all bacterial spores. Steam under pressure, hydrogen peroxide gas plasma, ethylene oxide gas and dry heat are the main validated sterilization processes for use in the healthcare facilities. Sterilization is essential for culture media, and critical items intended to enter the vascular system and sterile tissues such as vascular catheters and surgical instruments.

#### • Disinfection:

It is a process that eliminates most, if not all, pathogenic microorganisms except spores. Thus unlike sterilization, disinfection is not sporicidal. Disinfection is required for devices or equipment that do not penetrate tissues but used in contact with the skin (e.g., stethoscope diaphragm swabbed with (70%) alcohol) or mucous membranes (e.g., immersion of endoscope in 2% ortho-phthalaldehyde (OPA) disinfectant for 12 minutes).

#### • Disinfectant:

Usually a chemical agent (but sometimes a physical agent) that achieves disinfection. It refers to substances applied to inanimate objects.

محرر

Disinfectants may be categorized into 3 levels: high, intermediate and low:

**1. High level disinfectant:**

Germicide that kills all microbial pathogens except large numbers spores. Examples include OPA for endoscopes, hydrogen peroxide for contact lens, chlorine for blood spills.

**2. Intermediate level disinfectant:**

Germicide that kills all microbial pathogens except bacterial spores. Examples include isopropyl alcohol and iodophors.

**3. Low level disinfectant:**

Germicide that kills most vegetative bacteria (except tubercle bacilli) and lipid-enveloped and medium-sized viruses such as human immunodeficiency virus and hepatitis B virus e.g., quaternary ammonium compounds for disinfection of floors and food preparation areas.

• **Antiseptic:**

A chemical disinfectant which can be safely applied to skin and mucous membranes but not suitable for systemic administration. The term is used especially for preparations applied topically to living tissue (e.g., 70% isopropyl alcohol to prepare skin for injection, preoperative skin preparation with alcohol-based iodine compound in surgical operations).

• **Germicide:**

Agent that destroys microorganisms; may be virucide, bactericide, fungicide, sporicide and tuberculocide indicating the microorganisms the germicide kills. The term germicide includes both antiseptics and disinfectants. Antiseptics are germicides applied to living tissue and skin; disinfectants applied only to inanimate objects.

• **Sterilant:**

Chemical germicide that achieves sterilization

• **Cleaning (or precleaning):**

Removal of foreign material (organic or inorganic contaminants) from medical devices as part of decontamination process. It is usually done with water and soap, detergents or enzymatic products. Cleaning must always precede disinfection and sterilization.

• **Decontamination:**

Reduction of pathogenic microorganisms to a level at which items are safe to handle. Decontamination includes sterilization and all disinfection levels.

## MAIN METHODS OF DISINFECTION

1. **Chemical disinfectants** (see above). *high, intermediate, low.*
2. **Boiling water:** Boiling ( $100^{\circ}\text{C}$ ) for 20 minutes achieves high disinfection. It can be useful in emergencies if sterilizer is not available.
3. **Pasteurization:** Pasteurization of milk by heating at  $63^{\circ}\text{C}$  for 30 min. or at  $72^{\circ}\text{C}$  for 20 sec., followed by rapid cooling, destroys important pathogens e.g. *Mycobacterium tuberculosis*, *Brucella*, *Salmonella* and *Coxiella burnetti*.
4. **Thermal disinfection by hot water** can be performed in special washing machines e.g. for <sup>linen</sup> in hospital laundry, dishes and devices which cannot withstand higher temperature. *advised* *cloth*
5. **Ultraviolet radiation (UV):** UV can be artificially produced by mercury lamps. UV rays have weak penetration power and is used only for air and surface disinfection, e.g., laboratory safety cabinets.

## METHODS OF STERILIZATION

### I. Steam sterilization:

- It is the most safe and commonly used sterilization method.
- It is accomplished in an autoclave and uses moist heat in the form of saturated (dry) steam under pressure for a specified exposure time and at a specified temperature, as the sterilizing agent.
- Thus, there are four parameters of steam sterilization: steam, pressure, temperature, and time.
- The ideal steam for sterilization is saturated steam. It is essential to make steam saturated which means free of air because air acts as an insulator and hinders penetration.
- Pressure serves as a means to obtain the high temperatures necessary to quickly kill microorganisms.
- The two common steam-sterilizing temperatures are  $121^{\circ}\text{C}$  (maintained for a minimal exposure time 30 minutes) and  $132^{\circ}\text{C}$  (maintained for 4 minutes).
- As regard mode of action, moist heat destroys microorganisms by coagulation and denaturation of enzymes and structural proteins.
- Steam sterilization is nontoxic, inexpensive and rapidly heats and penetrates fabrics. It is the most widely used and the most reliable.



**Monitoring of steam sterilizers (autoclaves),** use following 3 monitors:

**1. Mechanical indicators:**

Using a printout or graph that monitors the time, temperature and pressure of the sterilization cycle.

**2. Chemical indicators or integrators:**

Chemically impregnated paper strips that must be used with each sterilization cycle to monitor the temperature or time and temperature. Visible colour changes occur at specified temperature and time.

**3. Biological indicators:**

Paper strips impregnated with the spores of *Geobacillus stearothermophilus*. The biological indicators are placed at the coldest point of the chamber. After finishing the cycle of sterilization, spore strips are incubated in a fluid medium at 37°C for 48h. Absence of bacterial growth indicates an efficient sterilization cycle.

## **II. Low temperature sterilization methods:**

### **1. Hydrogen peroxide gas plasma**

- Gas plasmas have been referred to as the fourth state of matter (i.e., liquids, solids, gases, and gas plasmas). Gas plasmas are generated in an enclosed chamber under deep vacuum using radio frequency or microwave energy to excite the gas molecules and produce charged particles, many of which are in the form of free radicals.
- The free radicals interact with essential cell components (e.g., enzymes, nucleic acids) and thereby disrupt the metabolism of microorganisms, in addition to the direct inactivation by hydrogen peroxide.
- Total time of sterilization cycle is about 50 minutes.
- Medical materials and devices that cannot tolerate high temperatures and humidity, such as some plastics, electrical devices, and corrosion-susceptible metal alloys, can be sterilized by this method.
- *G. stearothermophilus* (formerly *Bacillus stearothermophilus*) spores are used as a biological indicator to monitor efficiency of the sterilization process.

### **2. Ethylene oxide gas sterilization**

- Exposure time is long and varies from 3 to 6 hours.
- The method is expensive with probable toxicity.
- It can be used for instruments that cannot be subjected to steam.
- *Bacillus atrophaeus* (formerly *B subtilis*) spores are used as a biological indicator.

### **3. Peracetic acid sterilization**

- It is used to sterilize medical, surgical, and dental instruments (e.g., endoscopes, arthroscopes).
- Peracetic acid denatures proteins, disrupts cell wall, and oxidizes proteins and enzymes of microbes.

### III. Dry heat sterilization:

includes the following forms:

1. **Incineration:** is particularly applicable for dead animal bodies, infectious hospital waste such as used surgical dressings, needles....etc.
2. **Red heat:** Inoculating wires, loops and points of forceps are sterilized by holding them in the flame until they are red hot.
3. **Dry Heat Sterilizers or hot air ovens**
  - The method employs dry hot air as the sterilizing agent.
  - The most common time-temperature relationships are 170°C for 60 minutes, 160°C for 120 minutes, and 150°C for 150 minutes.
  - *Bacillus atrophaeus* spores should be used as a biological indicator.
  - Mode of action, killing is due to oxidation of the microbial cell constituents
  - This method is used for materials that might be damaged by moist heat (e.g., powders, petroleum products, sharp instruments).
  - The advantages for dry heat include the following: it is nontoxic, relatively inexpensive, and it is noncorrosive for metal and sharp instruments.
  - The disadvantages are the slow rate of heat penetration, and time-consuming and the high temperatures are not suitable for most materials.

### IV. Other sterilization methods

#### 1. Ionizing radiation:

- Sterilization by ionizing radiation can be obtained by cobalt 60 gamma rays or electron accelerators ( $\beta$ -rays).
- Ionizing radiation has a high penetrating power and is, therefore, used for sterilization of prepacked heat-sensitive items such as bone grafts, surgical sutures, disposable plastic syringes, gloves, catheters and plastic Petri dishes.
- *Bacillus pumilus* spores are used as a biological indicator to monitor efficiency of radiation sterilization.

#### 2. Filtration:

- A process used to remove bacteria from thermolabile pharmaceutical fluids (antibiotic solutions, hormones, vitamins) that cannot be purified by any other means. Fluids can be rendered free of bacteria by passage through bacterial membrane filters with pore size as small as 0.22  $\mu$ m.
- Filters can also be used to remove microorganisms from air supplied to critical areas such as operating rooms, drug factories and laboratory biosafety cabinets. Such filters are known as high efficiency particulate air (HEPA) filters which can provide sterile air at the filter face.
- The endospore producing *Serratia marcescens* may be used to test the efficiency of bacterial membrane filters, while spores of the fungus *Aspergillus* may be used to test the efficiency of HEPA filters.

**3. Ozone:**

- Ozone ( $O_3$ ) consists of  $O_2$  with a loosely bonded third oxygen atom that makes ozone a powerful oxidant that destroys microorganisms.
- Ozone has been used for years as a drinking water disinfectant.

**4. Formaldehyde Steam:**

- Low-temperature sterilization method that involves use of formalin, which is vaporized into formaldehyde gas.
- The method may be used in healthcare facilities to sterilize heat-sensitive medical equipment such as the mechanical ventilator and incubators for neonates.
- Unfortunately formaldehyde is a mutagen and a potential human carcinogen, therefore must be regulated and fully contained to guarantee the permissible exposure limit of healthcare workers for formaldehyde.

**5. Infrared radiation****MCQs:****1- One of the following statements is CORRECT:**

- ☒ a- Sterilization is complete removal or inactivation of all forms of microbial life.
- b- Disinfection is elimination of all pathogenic organisms including spores.  $\times$
- c- Low level disinfection is effective against *Mycobacterium tuberculosis*.  $\times$
- d- Antiseptics are chemical disinfectants applied to surfaces and floors.  $\times$
- e- High level disinfection is enough for surgical instruments and needles.  $\checkmark$

**2- Pasteurization:**

- a- Is generally performed at  $87^\circ\text{C}$  for 30 minutes
- ☒ b- Can destroy important pathogenic organisms
- c- Is a method of sterilization  $\times$
- d- Is done by hot water at temperatures higher than  $100^\circ\text{C}$   $\times$
- e- Cannot destroy *Mycobacterium tuberculosis*  $\times$

**3- Regarding hot air oven:**

- a- It is used to sterilize powders and petroleum products.
- b- The sterilizing agent is moist heat.
- c- It has a corroding effect.
- d- It doesn't necessitate prolonged exposure.
- e- It is characterized by rapid and even penetration of heat into the materials to be sterilized.

**4- Regarding biological indicators for monitoring autoclaves:**

- a- They are placed at the hottest part of the chamber.
- b- They change their colour at  $121^\circ\text{C}$
- ☒ c- They are paper strips containing the spores of *G. stearothermophilus*.
- d- Presence of bacterial growth indicates an efficient sterilization cycle.
- e- They are checked for colour change at the end of each sterilization cycle.



## BACTERIAL PATHOGENESIS

### ILOs:

By the end of this chapter the student should be able to:

- Contrast the terms infection and disease
- Explain mutual relationship between bacteria and host
- Explain infectious process
- Illustrate Koch's postulate
- Contrast the terms pathogenicity and virulence
- List virulence factors of bacteria
- Contrast exotoxins and endotoxins

**Infection** is a process by which the organism enters into a relationship with the host. Although microbial infections occur frequently, most infections end without occurrence of pathological changes and thus are not manifested as clinical disease. These infections are termed **subclinical**, **silent** or **abortive** infections.

The outcome of bacterial infections depends on the mutual relationship between bacteria and host. Accordingly, bacteria could be classified into:

1. **Saprophytic bacteria**: are those which live freely in nature, on decaying organic matter, in soil or water. They do not require a living host.
2. **Parasitic bacteria**: are those which live on or in a living host. They are classified according to their relation to the host into:
  - a- **Pathogenic**: Bacteria capable of causing disease.
  - b- **Non-pathogenic (commensals)**: Bacteria that do not cause disease, and are part of the normal flora.
  - c- **Opportunistic pathogens**: These are potentially pathogenic bacteria that do not cause disease under normal conditions but can cause disease in immunocompromised patients, or when they find their way to another site other than their normal habitat. Many of these opportunistic pathogens are originally commensals.

### Stages of the Infectious Process

1. **Source of infection** which may be man (case or carrier), animal or soil.
2. **Mode of transmission** e.g. droplet inhalation, ingestion, injection, insects, contact and transplacental.
3. **Portal of entry** e.g. respiratory tract, gastrointestinal tract, skin...etc. The organism then starts to multiply within the host causing tissue damage (disease).
4. **Portal of exit** e.g. urine, stools, blood, respiratory or genital discharge, from which the organism is transmitted to a new host.

### Koch's postulates

These are criteria that were proposed by Koch in order to determine if the organism isolated from the patient actually caused the disease, i.e. these criteria must be satisfied to confirm the causal role of an organism. These criteria are as follows:

1. The organism must be isolated from every patient with the disease.
2. The organism must be isolated free from all other organisms and grown in pure culture *in vitro*.
3. The pure organism must cause the disease in a healthy, susceptible animal.
4. The organism must be recovered from the inoculated animal.

The outcome of infection depends on the interaction between microbial factors (**virulence**) and host resistance factors (**immunity**).

## Microbial Virulence

While **pathogenicity** is a qualitative description of a species of bacteria denoting ability to produce disease, **virulence** is a quantitative character (degree of pathogenicity) of a strain belonging to a pathogenic species. Virulence is genetically determined by genes carried on plasmids, phages, pathogenicity islands and chromosomes.

### Virulence Factors of Bacteria

A virulence factor is either a structure (e.g. capsule) or a product (e.g. toxins) that enables the organism to cause disease.

#### A- Adherence factors

They enable bacteria to attach to the host surfaces, thus contributing to the establishment of the infection. For example:

1. The fimbriae of *Neisseria gonorrhoeae* and *E. coli* help the attachment of these organisms to the urinary tract epithelium.
2. The glycocalyx of *Staphylococcus epidermidis* and certain viridans streptococci allows the organisms to adhere strongly to the heart valves.

Mutants that lack these factors are often avirulent.

#### B- Invasion factors

Invasion of tissue followed by inflammation is one of the main mechanisms by which bacteria can cause disease. This invasion is helped by:

##### 1. Enzymes

- a- Collagenase and hyaluronidase which degrade collagen and hyaluronic acid and allow the bacteria to spread through subcutaneous tissues.
- b- Immunoglobulin A protease which degrades IgA.
- c- Leukocidin which can destroy both polymorphonuclear leucocytes and macrophages. *neutrophils.*
- d- Deoxyribonuclease that breaks down DNA.
- e- Lecithinase that breaks down lecithin of cell membrane.



## 2. Antiphagocytic factors

- a- Capsule: The capsule prevents the phagocytes from attachment to the bacteria, e.g. *Strept. pneumoniae*.
- b- Cell wall proteins of Gram-positive cocci, such as the M protein of *Strept. pyogenes* and protein A of *Staph. aureus*.
- c- Coagulase: It accelerates the formation of a fibrin clot from fibrinogen. This clot can protect bacteria from phagocytosis, e.g. *Staph. aureus*.

## C- Toxin production

Toxin production is another mechanism by which bacteria can produce disease. Bacterial toxins are either exotoxins or endotoxins (Table 4).

**Table (4): Comparison of the main features of exotoxins and endotoxins:**

	Exotoxins	Endotoxins
<b>Source</b>	Secreted by living organisms both Gram-positive (mainly) and Gram-negative	Integral part of the cell wall of Gram-negative organisms. Liberated upon cell disintegration
<b>Coding genes</b>	Encoded by chromosomes, plasmids, bacteriophages or PAI	Encoded by genes on the Chromosome
<b>Examples</b>	<i>C. diphtheriae</i> (phage) <i>Cl. tetani</i> (plasmid) <i>B. pertussis</i> (chromosome) <i>H. pylori</i> (PAI)	<i>E.coli</i> and meningococcal Endotoxins
<b>Nature</b>	Protein	Lipopolysaccharide (lipid A)
<b>Antigenicity</b>	Highly antigenic	Poorly antigenic
<b>Heat stability</b>	Unstable to temp. above 60°C	Stable to temp. above 60°C for several hours
<b>Detoxification</b>	Can be converted into toxoid*	Can not
<b>Specificity</b>	Every toxin has specific action	Same generalized effect (non-specific action), all give fever and shock
<b>Toxicity</b>	High	Low

\* Treatment of exotoxin with formalin (or other agents) removes its toxicity and retains its antigenicity converting it into toxoid, that can be used for immunization.

## MCQs:

### 1- Opportunistic pathogens:

- a- Are never the cause of a clinical infection
- b- Are usually highly pathogenic
- c- Are rarely part of the normal flora
- ☒ d- Cause disease mainly in immunocompromised individuals
- e- Are resistant to killing by steam sterilization



- 2- Exotoxins have the following characters, EXCEPT:
- a- They may be encoded by genes on the chromosome. ✓
  - b- They can be converted to toxoids. ✓
  - c- They have specific action. ✓
  - d- They are polypeptides. ✓
  - ☒ e- They are heat stable.
- 3- Endotoxins:
- a- Are secreted mainly by Gram-positive bacteria
  - b- Are highly antigenic
  - ☒ c- Are stable at temperatures above 60°C
  - d- Can be converted into toxoid
  - e- Have specific action

# **GENERAL VIROLOGY**





## GENERAL VIROLOGY

### **ILOs:**

**By the end of this chapter, the student should be able to:**

- Compare and differentiate between viruses and bacteria
- Identify and illustrate the structure and composition of viruses
- Classify viruses
- Summarize the steps of virus replication
- Explain the pathogenesis of viral diseases
- Compare between local and systemic viral infections
- Identify the laboratory diagnosis of viral infections
- List the different types of antiviral drugs, their mechanism of action and clinical use

Viruses are one of the smallest infectious agents. They are obligatory intracellular parasites because they have no metabolic activity.

### **Viruses can infect all organisms in nature:**

1. Bacteriophages: are bacterial viruses.
2. Plant viruses: include complete viruses and viroids.
3. Animal viruses: infect insects or vertebrates including man.

### **Viruses differ from bacteria in the following:**

1. Viruses are very small in size, ranging from 20-300 nm. Therefore:
  - They can only be seen under the electron microscope (except poxviruses).
  - They can pass through bacterial filters.
  - They need ultracentrifugation for sedimentation.
2. Viruses contain only one type of nucleic acid (DNA or RNA), never both.
3. They are obligatory intracellular parasites (can only replicate inside living cells) and do not divide by binary fission.
4. They cannot be cultivated in the laboratory on artificial culture media; however, they can be grown on tissue culture.
5. They are not susceptible to antibacterial antibiotics.

## Structure and Composition of Viruses

The typical complete virus particle, called **virion**, consists of a **genome** of either DNA or RNA, surrounded by a **capsid** (protein coat). The nucleic acid and the protein coat are called **nucleocapsid**. Some viruses, called **enveloped viruses**, have an outer lipid-containing **envelope** whereas others are **non-enveloped** (naked) (Fig. 11).

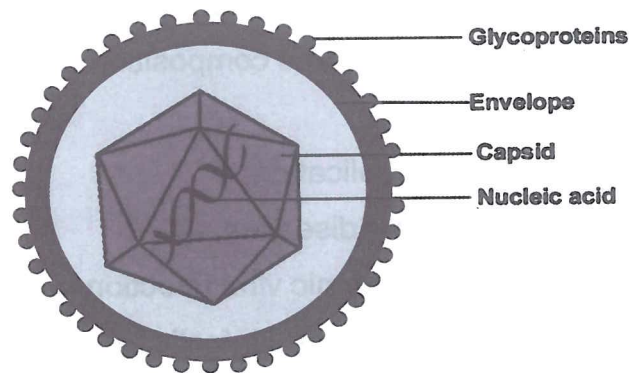


Fig. (11): The components of the complete virus particle (virion).

### Viral nucleic acid (genome)

- It is the genetic material of a virus, which may be either RNA or DNA.
- Most DNA viruses are double-stranded (ds) while most RNA viruses are single-stranded (ss). The viral ssRNA may be positive sense strand (+sense) or negative sense strand (-sense).
- It is responsible for virulence, i.e. it is the infectious part of the virus.

### Viral capsid

- Viral capsid is composed of many small protein subunits called **capsomers**.
- It has the following functions:
  1. It protects the nucleic acid (genome) against harmful environmental factors.
  2. It mediates attachment to host cell (in non-enveloped viruses).
  3. It is responsible for the viral symmetry (or morphology) which may be:
    - **Icosahedral (many sided) symmetry** (Fig. 12a): Icosahedral or isomeric or cubic viruses resemble a crystal with 20 triangular facets and 12 corners. This includes all DNA viruses, except poxviruses (brick shaped), and some RNA viruses.
    - **Helical (coiled tubes) symmetry** (Fig. 12b): The viral nucleic acid is closely associated with the protein capsid forming a coil-shaped helical nucleocapsid. This includes many of RNA viruses, e.g. rabies virus.
    - **Complex symmetry**: Examples include the brick-shaped poxviruses (Fig.12c) or bacteriophages (Fig. 12d).



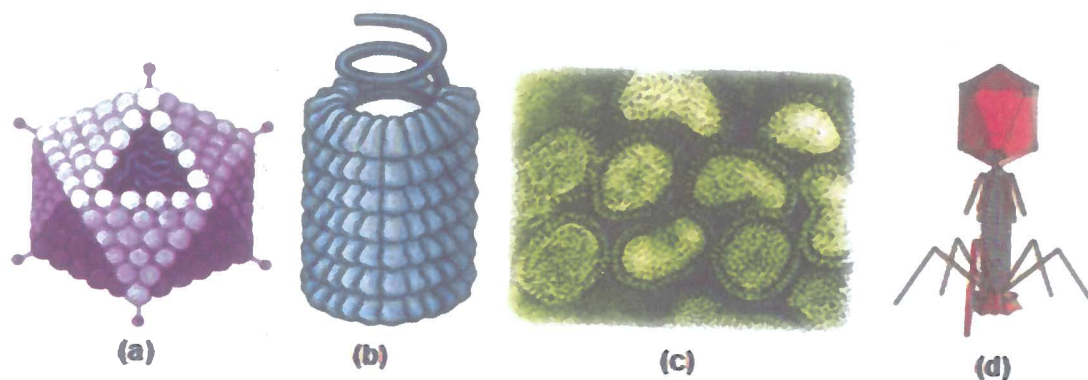


Fig. (12): Virus Symmetry

### Viral envelope

- It is a lipoprotein membrane composed of lipids, derived from host cell membrane during release by budding, and protein that is virus-specific.
- Frequently, the envelope may have glycoprotein spikes which are the organ of attachment of the enveloped virus to host cell receptors. Therefore, dissolving the envelope inhibits attachment and the virus loses its infectivity.
- Enveloped viruses are less stable, i.e. more easily inactivated than naked viruses. They are more sensitive to heat, drying, detergents and lipid solvents. Therefore, enveloped viruses, being unable to survive in the environment, are transmitted essentially by direct contact via blood and body fluids.

N.B.: The surface proteins of the virus, whether they are the capsid proteins (in naked viruses) or the glycoproteins (in enveloped viruses) are:

- responsible for attachment to host cell receptors
- the principal antigens against which the host elicits its immune response to viruses.

## Classification of Viruses

### A- Classification by symptomatology

It is the old classification based on diseases that viruses produce, i.e. tropism, e.g. neurotropic viruses, enteroviruses, .... etc.

### B- The hierarchical virus classification

The scheme classifying viruses into orders, families and subfamilies is based on:

1. Nature of the nucleic acid: RNA or DNA genome
2. Virus replication strategy
3. Symmetry of the capsid
4. Presence or absence of an envelope

Further classification is based on additional properties, e.g. antigenicity, host range and nucleic acid sequence.

### C- The Baltimore classification:

It is based on virus genome replication strategy. The central idea is that all viruses must generate positive strand mRNAs from their genomes, in order to produce proteins and replicate themselves. The precise mechanisms whereby this is achieved differ for each virus family.



## Virus Replication

Viruses are unable to replicate on their own because they lack the genes and enzymes necessary for energy production. Therefore, replication depends on living host cells and is directed by the viral genome to produce the virus components. Viral replication occurs in the following steps (Fig. 13):

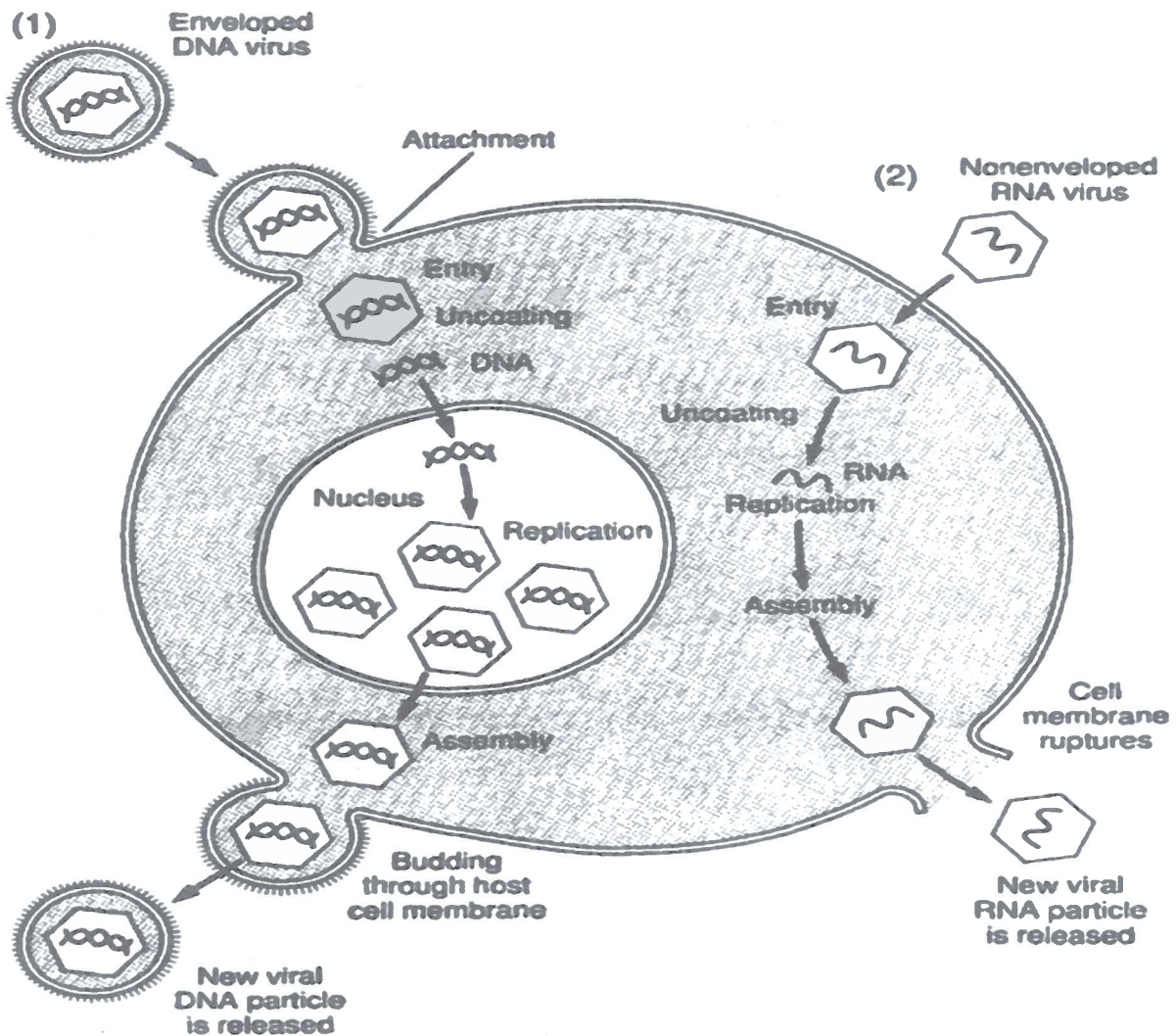


Fig. (13): Steps of viral replication

**1. Attachment or adsorption:** Adsorption of the virus occurs to specific receptor sites on the surface of the susceptible host cell. These interactions determine viral host range (e.g., human viruses and plant viruses) and tissue specificity or tropism (e.g. hepatotropic viruses and neurotropic viruses).

**2. Penetration:** The viruses may enter the host cells by either:  
 a- endocytosis in case of non-enveloped viruses, or  
 b- fusion of viral envelope with host cell membrane in case of enveloped viruses.

**3. Uncoating:** The nucleic acid is released from the capsid by the action of cellular enzymes and becomes available for replication.

#### 4. Synthesis of viral components

##### A) Synthesis of viral proteins:

###### Transcription:

Viruses must first synthesize virus-specific messenger RNA (mRNA) to synthesize virus specific proteins.

Transcription of mRNA varies according to the type of viral nucleic acid whether DNA or RNA, ds or ss, positive or negative sense strand, as follows:

a) **DNA viruses:** mRNA can be transcribed from the negative sense strand using the host's DNA-dependent RNA polymerase.

b) **RNA viruses:** There is no host cell RNA polymerase that can use the viral RNA as a template for synthesis of mRNA.

RNA viruses fall into 4 groups according to the strategy for synthesizing mRNA (Fig.14a):

- In dsRNA viruses, the negative sense strand is transcribed by viral RNA-dependent RNA polymerase into mRNA.
- In ssRNA viruses there are 3 distinct routes to the formation of mRNA:
  - i. The strand with positive sense acts directly as mRNA.
  - ii. The strand with negative sense must first be transcribed, using viral RNA-dependent RNA polymerase, into positive sense strand which can then act as mRNA.
  - iii. In retroviruses, the positive ssRNA is first made into a negative sense ssDNA using the viral reverse transcriptase. Then dsDNA is formed by the host DNA-dependent DNA polymerase. This dsDNA enters the nucleus and is either:
    - transcribed by host's DNA-dependent RNA polymerase into mRNA or
    - integrated in host cell genome causing transformation.

###### Translation:

Once the viral mRNA is transcribed, it is translated using host ribosomes to synthesize viral proteins.

##### B) Synthesis of viral nucleic acid:

Replication of the viral genome requires the synthesis of a strand with a complementary base sequence, which serves as the template for the synthesis of several copies of the original viral genome. (Fig.14 b)

**5. Assembly:** The newly synthesized protein coats enclose the replicated nucleic acids to form mature viruses (virions). This occurs either in the nucleus of the host cell, e.g. herpes viruses or in the cytoplasm, e.g. polioviruses.

**6. Release:** The new viruses are released either by:

- lysis of host cell in case of non-enveloped viruses, e.g. poliovirus or
- budding through the cell membrane in case of enveloped viruses, e.g. HIV.

##### **N.B.:**

- **Eclipse** is the time from uncoating until assembly of mature viruses. During this phase, no infectious viruses can be detected in the host cell.
- Some viruses do not initiate synthesis and remain **latent** within the host cell for variable periods.



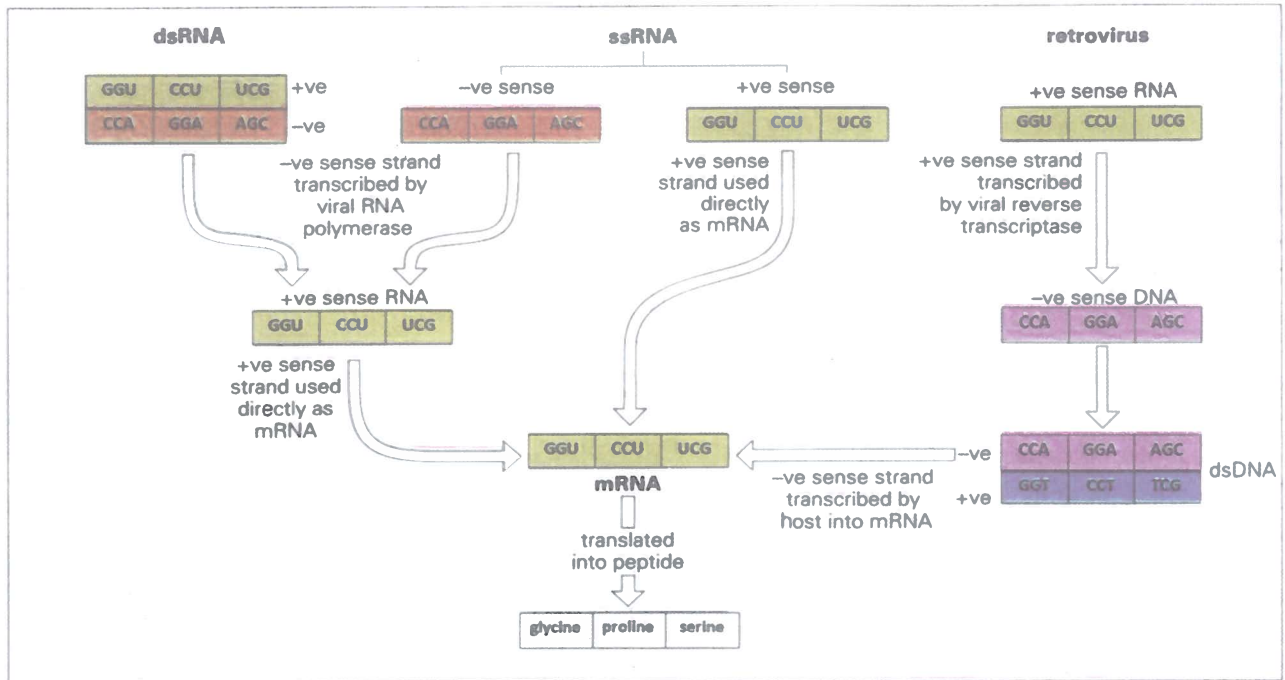


Fig. 14a: Transcription in RNA viruses

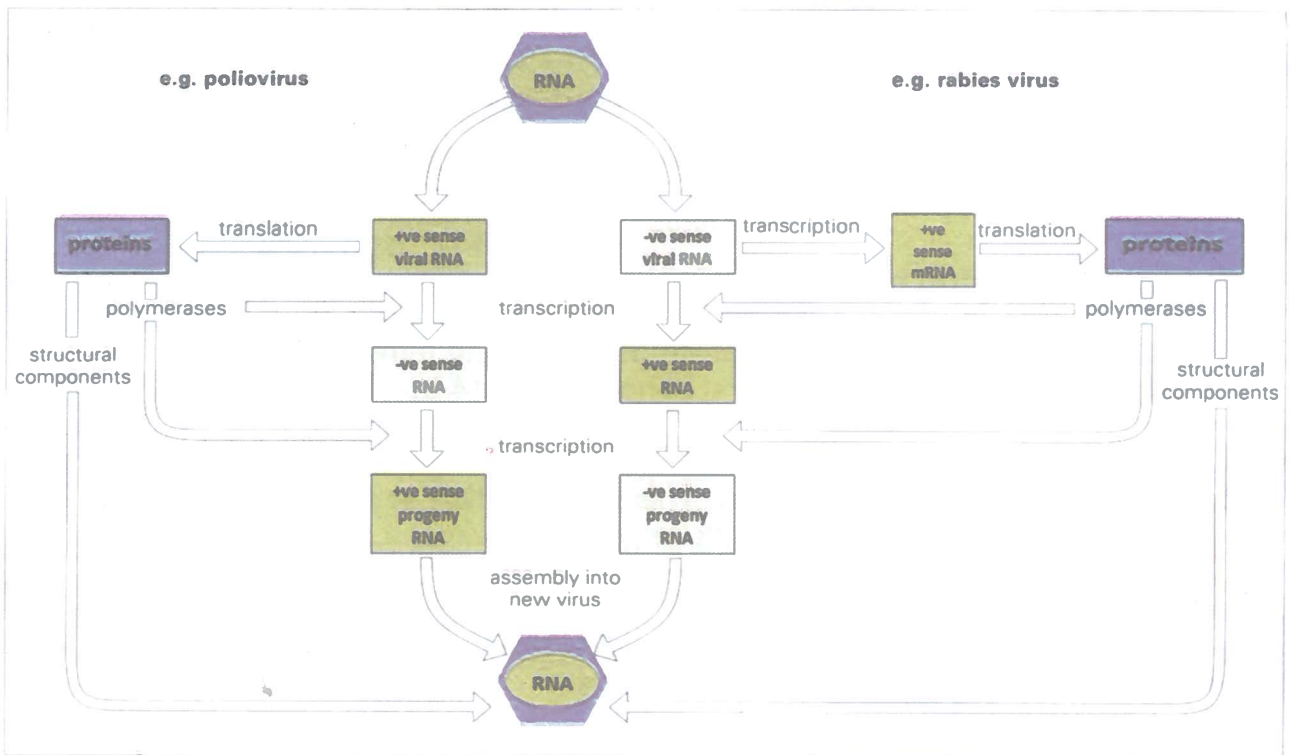


Fig.14b: Replication of the viral genome



## Pathogenesis of Viral Diseases

### Entry of viruses

- Viruses enter the body either by inhalation (respiratory tract), ingestion (gastrointestinal tract), contact (urogenital system) or through skin (injections, blood transfusion, insect and animal bites).
- Viral infection may be:
  - Local infection: where the virus produces disease at the portal of entry.
  - Systemic or deep viral infections: where the virus spreads to distant organs either via the blood (viraemia), or by other means, e.g. along nerves (Table 5).

**Table (5):** Differences between local and systemic viral infections.

	<b>Local infections</b>	<b>Systemic infections</b>
<b>Example</b>	Common cold (e.g. rhinovirus infection)	Measles
<b>Site of pathology</b>	Portal of entry	At distant sites
<b>Incubation period</b>	Relatively short	Relatively long
<b>Viraemia</b>	Absent	Present
<b>Duration of immunity</b>	Usually short	Usually lifelong
<b>Involved immunoglobulin</b>	Secretory IgA	IgM and IgG

### Fate of viral infections

- 1. Inapparent or subclinical viral infections:** Viral infection without overt signs and symptoms.
- 2. Apparent infections (disease):** Local or systemic viral infections with the appearance of clinical signs and symptoms.
- 3. Persistent viral infections (chronic):** In this form, the virus is continuously detected with mild or no clinical symptoms, e.g. chronic hepatitis B.
- 4. Latent viral infections:** The virus persists in a dormant form and may flare up intermittently to produce disease, e.g. herpes viruses.
- 5. Slow virus infections:** Virus infections with long incubation periods (months or years). They are caused by two types of infectious agents:
  1. Conventional viruses, e.g. a variant of measles virus which causes subacute sclerosing panencephalitis (SSPE).
  2. Unconventional agents (prions).

## Laboratory Diagnosis of Viral Infections

The laboratory diagnosis of viral infection involves 2 main diagnostic methods:

**A- Direct methods:** which depend either on the detection of viruses and/or their components in the patient's specimens, or on isolation of viruses.

**B- Indirect methods:** which depend mainly on the detection of antibodies against the suspected virus in the patient's serum, or on skin tests.

The different techniques used in diagnosis of viral infections are discussed in "Practical Microbiology and Immunology".

## Treatment of Viral Infections

Viruses can not be treated with antibiotics because they lack the structural targets on which antibiotics can act. Viruses are obligate intracellular parasites, so antiviral drugs must selectively inhibit viral replication without causing damage to host cells. The number of antiviral drugs is little compared to antibacterial drugs (Table 6):

**Table (6):** Selected antiviral drugs and their mechanism of action and clinical use

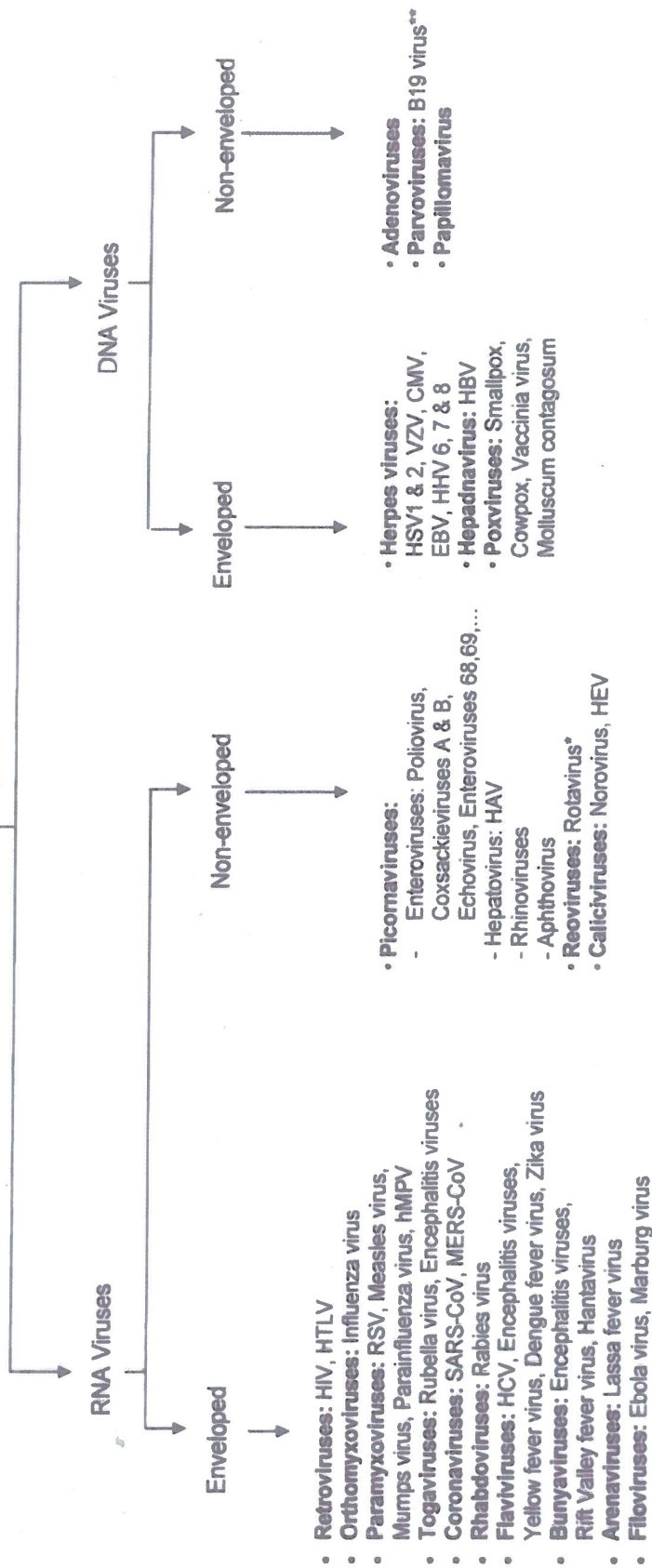
Mechanism of action	Antiviral drugs	Virus infections
<b>I. Fusion inhibitors</b> (block virus entry)	Fuzeon (enfuvirtide)	HIV
<b>II. Uncoating inhibitors</b> (inhibit virus uncoating)	1. Amantadine	Influenza A virus
	2. Rimantadine	Influenza A virus
<b>III. Neuraminidase inhibitors</b> (interfere with release of virus from infected cells)	1. Oseltamivir (Tamiflu)	<ul style="list-style-type: none"> <li>▪ Influenza A virus</li> <li>▪ Influenza B virus</li> </ul>
	2. Zanamivir	<ul style="list-style-type: none"> <li>▪ Influenza A virus</li> <li>▪ Influenza B virus</li> </ul>
<b>IV. Nucleoside analogues that inhibit DNA polymerase;</b> (inhibit DNA synthesis)	1. Acyclovir	<ul style="list-style-type: none"> <li>▪ <b>Topical:</b> herpes simplex virus type 1 and 2 and varicella-zoster virus</li> <li>▪ <b>Parenteral:</b> HBV</li> </ul>
	2. Ganciclovir	Cytomegalovirus
	3. Vidarabine	Herpes viruses, HBV
	4. Iododeoxyuridine	herpetic keratoconjunctivitis
<b>V. Inhibitors of mRNA synthesis</b>	Ribavirin	Respiratory syncytial virus, influenza B virus, HCV
<b>VI. Nucleoside analogues that inhibit reverse transcriptase enzyme</b>	1. Azidothymidine	HIV
	2. Zalcitabine	HIV
	3. Lamivudine	HIV & HBV
	4. Stavudine	HIV
<b>VII. Protease inhibitors</b> they inhibit cleavage of precursors polypeptide	1. Indinavir	HIV
	2. Ritonavir	HIV
	3. Saquinavir	HIV
<b>VIII. Inhibitors of viral protein synthesis</b>	1. Methisazone	Poxviruses (smallpox)
	2. Interferons	<ul style="list-style-type: none"> <li>▪ HBV and HCV</li> <li>▪ Human papilloma virus</li> </ul>

**MCQs:**

- 1- Viruses differ from bacteria in the following **EXCEPT**:
  - a- They are very small in size.
  - b- They contain two types of nucleic acid.
  - c- They are obligatory intracellular parasites.
  - d- They need ultra-centrifugation for their sedimentation.
  - e- They can be seen only by the electron microscope.
  
- 2- All of the following is true concerning viral capsid **EXCEPT**:
  - a- It is formed of capsomers.
  - b- It is protein in nature.
  - c- It is responsible for viral symmetry.
  - d- It is the infectious part of the virus.
  - e- It protects the nucleic acid.
  
- 3- Local viral infections are characterized by:
  - a- Long incubation period
  - b- Short duration of immunity
  - c- Insignificant role of sIgA
  - d- Important role of IgM and IgG
  - e- A stage of viraemia



## Classification of Viruses



### N.B.:

- Hepatitis viruses (A-E) belong to different families.
- Arboviruses include members in *Flavi*-, *Toga*- and *Bunyaviridae* families.
- Roboviruses include members in *Bunyaviridae* and *Arenaviridae* families.
- Tumour viruses are present among different families

\* Rotavirus is the only double-stranded RNA virus.

\*\* Parvoviruses are the only single-stranded DNA viruses.

# **GENERAL MYCOLOGY**





## GENERAL MYCOLOGY

### ILOs:

**By the end of this chapter the student should be able to:**

- Explain the eukaryotic nature of fungi & identify the differences between fungi & bacteria
- Describe the morphological forms of fungi
- Outline the clinical classification of fungal infections
- Describe the pathogenesis of fungal infections
- List examples of antifungal drugs

Mycology is the study of fungi. Fungi differ from bacteria in the following (Table 7):

**Table (7): Comparison of fungi and bacteria**

Feature	Fungi	Bacteria
Size	Larger	Smaller
Nucleus	Eukaryotic	Prokaryotic
Mitochondria	Present	Absent
Ergosterol in cytoplasmic membrane	Present	Absent*
Cell wall content	Chitin	Peptidoglycan
Spores	For reproduction**	For survival
Metabolism	- Heterotrophic - No obligate anaerobes	- Hetero- and autotrophic - Many obligate anaerobes

\* *Mycoplasma* is the only bacteria that contains cholesterol in the cytoplasmic membrane

\*\*Reproduction may be by both sexual (meiotic) or asexual (mitotic) spores

## Morphological Forms:

### 1- Moulds:

- They consist of long filaments (hyphae) which may be:
  - Septate (with cross walls) (Fig. 15)
  - Non-septate (without cross walls).
- They grow by branching and tip elongation forming a mass called mycelium on culture media (Fig. 16)
- Examples include *Aspergillus*, *Penicillium* and the dermatophytes.

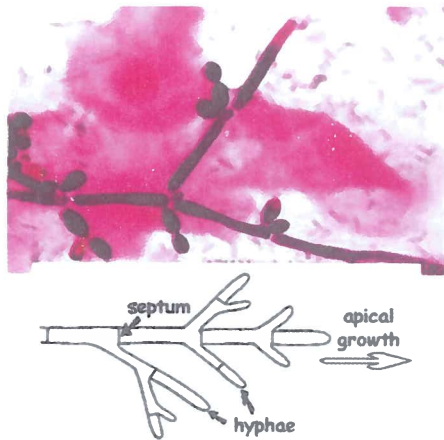


Fig. (15): Filamentous fungi

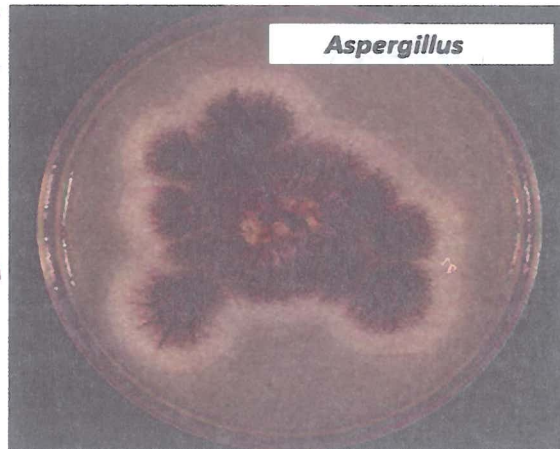


Fig. (16): Filamentous colonies

### 2- Yeasts:

- They grow as single cells (round or oval) (Fig. 17).
- They reproduce by budding and may form pseudohyphae (hyphae with constrictions; sausage-like chain) (Fig. 17).
- Examples include *Candida* and *Cryptococcus*.

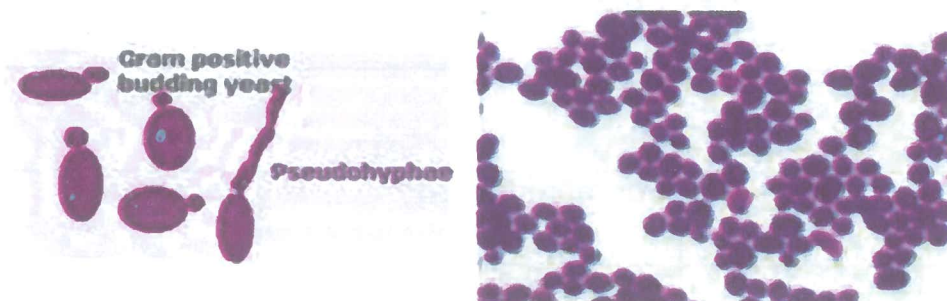


Fig. (17): Yeasts

N.B.: **Dimorphic fungi** (e.g., *Histoplasma*) are those that can switch between the previous two forms depending on the temperature:

- At room temperature: they grow as moulds (hyphae).
- At body temperature: they grow as yeasts.

## Clinical Classification:

### A- Mycotic infections

1. **Superficial mycoses:** affecting the keratinized layer of the skin, e.g. *Pityriasis versicolor*.
2. **Cutaneous mycoses:** affecting the deep layers of the skin, e.g. candida and dermatophytes.
3. **Subcutaneous mycoses:** in which fungi present in the soil are implanted in the subcutaneous tissue by trauma, e.g. mycetoma.
4. **Deep (systemic) mycoses:** affecting internal organs. These fungi fall in two groups:
  - "True pathogens" infecting normal healthy individuals, e.g., *Histoplasma* and *Blastomyces*.
  - "Opportunistic pathogens" infecting immunocompromised individuals, e.g., *Pneumocystis*, *Cryptococcus* and *Candida*.

**B- Mycotoxicosis:** It is produced by consumption of food containing fungal toxins, e.g.:

- Mushroom poisoning causes damage to liver, kidney and bone marrow.
- Aflatoxin of *Aspergillus flavus* may cause chronic liver damage and cancer.

**C- Allergic disorders:** Spores of free-living fungi as *Aspergillus* may be the allergen in some cases of atopy (asthma, hay fever, urticaria ... etc.).

## Pathogenesis

- Infection with certain systemic fungi (e.g., *Histoplasma*) elicits a granulomatous host defense response (composed of macrophages and helper T cells).
- Infection with other fungi (notably *Aspergillus*) elicits a pyogenic response (composed of neutrophils).

## Diagnosis of fungal infections

The laboratory diagnosis of fungal infection involves 2 main diagnostic methods:

**A- Direct methods:** which depend either on the detection of fungi and/or their antigens in the patient's specimens, or on isolation of fungi.

**B- Indirect methods:** which depend mainly on the detection of serum antibodies against the suspected fungus in systemic mycosis or, less frequently, on skin tests.

The different methods used in diagnosis of fungal infections are discussed in "Practical Microbiology and Immunology."



**Antifungal Drugs:**

- Because fungi are eukaryotes, the range of non-toxic systemically active antifungal drugs is still limited.
- The selective toxicity of antifungal drugs is based on the presence of **ergosterol** in fungal cell membranes, in contrast to the cholesterol found in human cell membranes and the absence of sterols in bacterial cell membranes.
- The most commonly used drugs are amphotericin B, mycostatin (nystatin) and azole drugs (e.g., fluconazole, ketoconazole and itraconazole).

**MCQs:**

- 1- Fungi have the following characters **EXCEPT**:
- a- They replicate sexually and asexually.
  - b- They are eukaryotic.
  - c- They have ergosterol in the cell membrane.
  - d- They are heterotrophic.
  - e- They are susceptible to antibacterial agents.

# ESSENTIAL MEDICAL MICROBIOLOGY and IMMUNOLOGY



Volume II





**ESSENTIAL  
MEDICAL MICROBIOLOGY  
AND IMMUNOLOGY**

**VOLUME II**  
**Immunology**

**Eighth Edition**

**By**

***Staff Members of  
Medical Microbiology and Immunology Department***

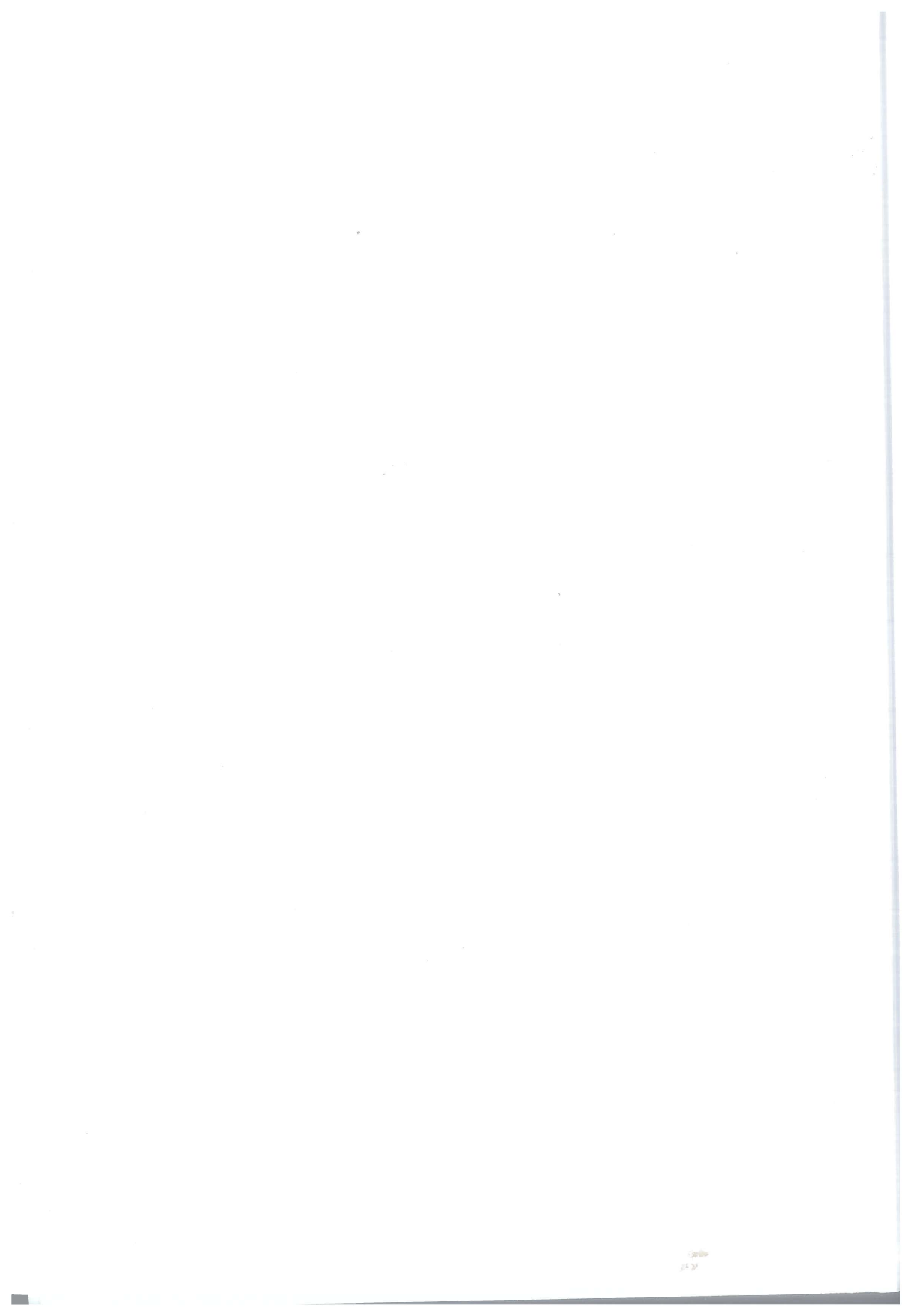
**Faculty of Medicine-Cairo University**  
**2018-2019**



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## OVERVIEW OF THE IMMUNE SYSTEM

### **ILOs:**

**By the end of this chapter the student should be able to:**

- Define innate and acquired (adaptive) immunity
- Describe the differences between innate and adaptive immunity
- Deduce the link between innate and adaptive responses
- List the components of the immune system
- Describe the role of each cell in the immune response
- Name cells of the innate and adaptive immune system
- Describe functions of cells of innate and adaptive immune system
- Explain different functions of macrophages
- Name the lymphoid organs
- Explain the terms 'primary' and 'secondary' lymphoid organs with examples
- Appreciate the significance of circulation of lymphocytes between blood and lymph
- Explain the term 'naïve lymphocytes'
- Appreciate the basis of immunological memory
- Explain the clonal selection theory
- List and describe the function of the main molecular components of the immune system including cellular and circulating factors
- Appreciate the consequences of failure of host defence mechanisms
- Appreciate the harmful immune responses
- Appreciate the different methods for manipulation of the immune system

Immunology is a relatively new science which has always been linked to the science of microbiology. This is because the main function of the immune system is to combat infections caused by different microbes. The immune system is composed of the leucocytes (granulocytes, monocytes and lymphocytes), as well as a number of lymphoid organs.

The immune response to infections includes two parts:

### I. Innate Immunity

It is immediately available to combat a wide variety of microorganisms without need for previous exposure. It includes many different resistance mechanisms such as barriers, chemicals and cells present in the body. It is very important at the beginning of an infection. However, it is not always successful in eliminating infectious organisms on its own. It does not increase with repeated exposure and cannot tell the difference between different pathogens.

Innate immunity depends mainly on the granulocytes (neutrophils, eosinophils and basophils), as well as monocytes/macrophages and natural killer (NK) cells.

### II. Acquired (Adaptive) Immunity

It is a specific immune response directed against a particular pathogen. It occurs during the lifetime of a person as an **adaptive** response to infection with that particular pathogen. It is associated with the development of **immunological memory**, so that in many cases it gives life-long protection against that same pathogen. It is very effective in combating infection, but there is a delay of a few days until it can start being effective.

Acquired immunity depends on T and B lymphocytes.

Table (1) shows a comparison between innate and acquired immunity.

**Table (1):** Comparison between innate and acquired immunity

	<b>Innate</b>	<b>Acquired</b>
<b>Onset of action</b>	Immediately after infection	Relatively delayed
<b>Main cells</b>	Granulocytes, monocytes/ macrophages & NK cells	B & T lymphocytes
<b>Memory</b>	Absent	Present
<b>Efficiency</b>	Less efficient	More efficient and improves with each exposure
<b>Specificity</b>	Non-specific: Present in all individuals, against all microorganisms	Specific: Occurs in a given person, against a particular pathogen

## The Components of the Immune System

### A. Cells of the Immune system

Following is a brief description of the role of each cell in the immune response:

- **Neutrophils** are phagocytic cells that are capable of engulfing and digesting foreign agents.
- **Eosinophils** are mainly of importance in defence against helminthic parasites.
- **Basophils** are found in the blood in very low concentrations and play a very important role in allergic reactions.
- **Mast cells** are similar to basophils but are found in tissues.



- **Monocytes/Macrophages:** Monocytes are present in the blood and continuously leave it to go to the tissues where they complete their maturation and become macrophages. They are phagocytic and have other properties important for the immune system.
- **Dendritic cells** are phagocytic cells of the innate immune system and act as an important bridge between it and the adaptive immune system.
- **Lymphocytes** include T and B lymphocytes as well as natural killer (NK) cells. Table (2) shows a comparison between T and B lymphocytes.

**Table (2)** Comparison between T and B lymphocytes:

	<b>T lymphocytes</b>	<b>B lymphocytes</b>
Production	Both are produced in the bone marrow	
Maturation	In the thymus	In the bone marrow
Count	Comprise 75% of peripheral blood lymphocytes (PBLs)	Comprise 10% of PBLs ( <i>less required to circulate because they secrete soluble antibodies</i> )
Functions	<p>There are two main kinds of T cells:</p> <ul style="list-style-type: none"> <li>• <b>Helper T (Th) cells</b>, whose main function is to secrete cytokines which help other cells of the immune system</li> <li>• <b>Cytotoxic T (Tc) cells</b>, whose main function is to kill abnormal body cells (infected cells and tumour cells)</li> </ul> <p>(The ratio of Th/Tc cells is 2:1)</p>	<p><b>Production of antibodies (immunoglobulins):</b></p> <p>When B cells become active, they change into plasma cells which secrete protein molecules called <b>antibodies</b>. These antibodies then circulate in the blood and have a very important role in many immune reactions.</p>
Receptors	<p>The T cell receptor:</p> <ul style="list-style-type: none"> <li>- Has one antigen-binding site</li> <li>- Cannot see antigens directly (Another cell called an antigen-presenting cell must "present" the antigen to T cells)</li> <li>- Is always a cell-surface molecule</li> <li>- Can detect antigens generated inside body cells</li> </ul>	<p>The B cell receptor:</p> <ul style="list-style-type: none"> <li>- Is a surface- immunoglobulin with two antigen-binding sites</li> <li>- Can see antigens directly</li> <li>- Can be secreted (as antibody in blood and body fluids)</li> <li>- Can detect antigens present outside body cells</li> </ul>

- **Natural Killer (NK) cells** are large granular lymphocytes which can be distinguished from B and T lymphocytes. They constitute 10-15% of peripheral blood lymphocytes. They are capable of killing abnormal body cells.

## B. The Lymphoid Organs

They are defined as organized tissues where lymphocytes interact with other non-lymphoid cells that are important either in their maturation or in starting an acquired immune response. They are divided into:

### I. Primary (central) lymphoid organs

This is where lymphocytes complete their maturation, becoming **mature** (adult) lymphocytes. They are:

- **The bone marrow:** where the B cells complete their maturation. (Thus, the bone marrow is not only the place of origin of all blood cells, but also acts as a central lymphoid organ).
- **The thymus:** where the T cells complete their maturation.

### II. Secondary (peripheral) lymphoid organs

They are the places where B and T lymphocytes can meet **antigens**, leading to activation of the lymphocytes. The secondary lymphoid organs include the spleen, lymph nodes and various mucosal associated lymphoid tissue (**MALT**):

- **The lymph nodes** are highly organized structures distributed all over the body.
- **The spleen** is mainly composed of red pulp, which is the site of RBC disposal, whereas the white pulp contains the lymphocytes.
- The gut-associated lymphoid tissue (**GALT**) includes the tonsils, adenoids, appendix and Peyer's patches.
- The bronchial-associated lymphoid tissue (**BALT**) includes similar but more diffusely organized collections of lymphocytes which protect the respiratory epithelium.
- Other mucosal sites contain similar collections.

## Circulation of Lymphocytes between Blood and Lymph: (Fig. 1)

1- Small B and T lymphocytes that have matured, but have not yet met antigen are called **naïve** lymphocytes. They leave the bone marrow and thymus, respectively, and circulate continually from the blood into secondary lymphoid organs, such as the lymph nodes, until they meet antigen or die.

2- Microbial antigens are drained from the site of infection through the afferent lymphatic vessels into the lymph nodes. Not any lymphocyte seeing an antigen will recognize it. This is because lymphocytes are very specific for the antigens they recognize. Lymphocytes which recognize a certain antigen undergo a series of changes which make them ready to start working against the antigen to which they are specific.

The changes which occur are:

- a. Activation:** They become lymphoblasts.
- b. Proliferation:** They multiply rapidly.
- c. Differentiation:** They change into **effector** cells, capable of being effective against the invading agent.

- B cells change into **plasma cells**, capable of secreting antibody.
- Cytotoxic T cells and helper T cells become **effector cytotoxic T cells** and **effector helper T cells** respectively.

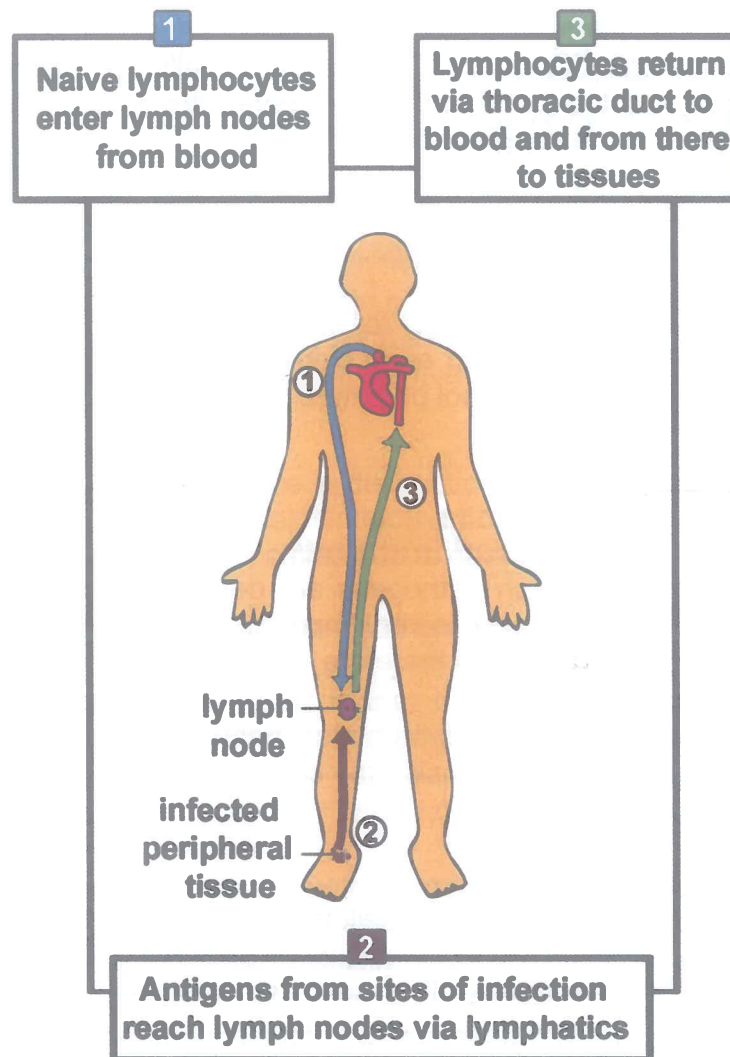


Fig. (1): Circulation of lymphocytes between blood and lymph

**3-** They now leave the lymph nodes through the efferent lymphatic vessels and return to the blood through the thoracic duct. From the blood, they reach the peripheral tissues where they start functioning to eliminate the specific infection which started their activation.

Some of the antigen-specific lymphocytes produced by these events remain after the antigen has been eliminated. These are called **memory cells** and are the basis of **immunological memory** which ensures a more rapid and effective response on a second meeting with the same pathogen and, therefore, gives long lasting immunity. Immunological memory is the most important biological sequence of the development of acquired immunity.



## The Clonal Selection Theory

- As mentioned above, T and B lymphocytes recognize antigen by very specific receptors present on their surface. Every naïve lymphocyte has a single type of receptor of certain specificity.
- Only those lymphocytes which meet the antigen which their receptors recognize will undergo activation, proliferation and differentiation.
- The result is a clone (family) of identical daughter cells, all having identical receptors, which can bind the same antigen wherever they find it. Thus, antigen specificity is maintained in the daughter cells.

*The clonal selection theory is so-called because a certain **clone** of cells is **selected** from the pool of naïve lymphocytes.*

- Lymphocytes having receptors specific for self molecules (a person's own molecules) are normally deleted at an early stage in lymphoid cell development and are, therefore, absent from the pool of mature lymphocytes.

## Effector Mechanisms of Acquired Immunity

These are the mechanisms by which pathogens are detected and destroyed in a successful acquired immune response. It is very important to remember that different pathogens have different lifestyles and, therefore, need different mechanisms for detection, recognition and destruction:

- B cells recognize antigen outside cells, where most bacteria are found.
- T cells can detect antigens generated inside the cell, as those belonging to viruses or intracellular bacteria.

### I. Effector Mechanisms of Antibodies

Antibodies work mainly to eliminate extracellular pathogens and their toxins. Antibodies are found in plasma and extracellular fluids. Immunity mediated by antibodies is called **humoral immunity**. It is so-called because body fluids were once known as **humors**.

In general, when antibodies bind to pathogens, they help in combating infection in many ways. They may block access of the pathogen to body cells. They may also start a process which eventually leads to lysis of the pathogen. All pathogens bound by antibody are eventually delivered to phagocytes for ingestion, degradation and removal from the body.

### II. Effector Mechanisms of T Cells

Pathogens are accessible to antibodies only in the blood and extracellular spaces. However, all viruses and some bacteria and parasites replicate inside cells, where they cannot be detected by antibodies. The destruction of these invaders is the function of the T lymphocytes. This is called **cell-mediated immunity**. In addition, T cells offer important help to B cells. The functions of T cells may be summarized as follows:

### 1. Cytotoxic T (Tc) cells

These recognize body cells infected with virus. Antigens from replicating viruses are displayed on the surface of infected cells, where they are recognized by the cytotoxic T cells. These cells directly kill the infected cells before viral replication is complete and new viruses are released to infect other cells. Tumour cells also carry abnormal antigens on their surface and are also killed by Tc cells.

### 2. Helper T (Th) cells

The function of these cells is mainly production of cytokines. There are two main kinds of helper T cells, grouped according to the type of cytokines they produce and the main cells they help:

#### a- T helper 1 (Th1) cells

These cells mainly secrete cytokines which help in **activation of macrophages**. This means making macrophages more capable of killing any bacteria inside them. This is very important because some bacteria, such as *M. tuberculosis*, after being ingested by macrophages, resist digestion and can survive for a long time inside.

#### b- T helper 2 (Th2) cells

These cells play a very important role in destruction of extracellular pathogens. They secrete certain cytokines which help in **activation of B cells**, so that they can become plasma cells and produce antibodies to deal with those pathogens.

It is important to remember that the decision whether Tc cells or Th cells are activated is not something which occurs by chance, but is decided by the type of pathogen and its lifestyle. The result is that the type of immune response which occurs is exactly suitable for combating that particular infection (see chapter 4).

## Harmful Immune Responses

The work of the immune system is usually of benefit to the body, as in elimination of infections or in dealing with tumour cells. On the other hand, some immune responses may be harmful. Many medically important diseases are associated with such immune responses:

- **Allergy:** inappropriate immune response against harmless antigens.
- **Autoimmune diseases:** immune response against the person's own antigens.
- **Graft rejection:** immune response against foreign tissues or organs transplanted in a person.

In all the above conditions, the immune response causes harm, not benefit. Thus, whether we consider an immune response harmful or beneficial depends not only on the response itself, but also on the nature of the antigen.

Even during a beneficial immune response against a pathogen, some tissue damage may occur due to the immune response.

## Failure of Host Defence Mechanisms

In some cases, a certain aspect or aspects of the immune system are deficient and the immune system is unable to free our body of infections. This is known as **immunodeficiency**. In very severe immunodeficiency diseases, adaptive immunity is completely eliminated and death occurs early in life. In less severe failures, there are specific recurrent infections. AIDS is a disease in which a virus infects T helper cells and destroys them. The person suffers from infections in which T helper cells normally play an important role in defence.

## Manipulation of the Immune System

- At present, the usual way to treat unwanted immune responses (allergy, autoimmune diseases and graft rejection) is by immunosuppressant drugs, which inhibit all immune responses, both destructive and beneficial. If, instead, it were possible to suppress only those lymphocytes responsible for unwanted (bad) responses, the disease would be cured without affecting protective (good) immune responses. This dream of **antigen-specific immunosuppression** is the subject of much research but has not been very successful till now.
- On the other hand, **antigen-specific immunostimulation** (stimulating the immune system against a particular antigen or group of antigens) has been more successful. This is done through **vaccination**, which involves introduction of pathogens or parts of them, in a harmless form, into the body, in order to stimulate the immune system to produce an immune response to that particular pathogen. The result is that when the person is later exposed to the same pathogen in its harmful form, he/she is ready to combat that infection because of immunological memory.

### MCQs:

- 1- Regarding innate immunity, all of the following is true **EXCEPT**:
  - a- It is present in all individuals since birth and at all times.
  - b- It is important at the beginning of infection.
  - c- It cannot tell the difference between pathogens.
  - d- It always eliminates infectious organisms successfully.
  - e- It involves mainly granulocytes.
- 2- Which of the following has an important role in parasitic infections?
  - a- Neutrophils
  - b- Mast cells
  - c- Eosinophils
  - d- Monocytes
  - e- Lymphocytes



- 3- Which of the following is a primary lymphoid organ:
- a- Bone marrow
  - b- Lymph nodes
  - c- Spleen
  - d- Tonsils
  - e- Peyer's patches
- 4- Naive lymphocytes are:
- a- Immature lymphocytes
  - b- Present only in the bone marrow and thymus
  - c- Responsible for immunological memory
  - d- Mature lymphocytes that have been activated by specific antigens
  - e- Mature lymphocytes that have not yet met antigen
- 5- Which statement about clonal selection theory is Correct:
- a- Every naive lymphocyte has many types of receptors.
  - b- A clone of cells can recognize different antigens.
  - c- Only lymphocytes which meet antigens they recognize are activated.
  - d- The T cell receptor has 2 antigen recognition sites.
  - e- The B cell receptor cannot recognize an antigen directly.
- Comparing BCR and TCR, which statement is TRUE?
- a- The B and T cell receptors become released into the surroundings.
  - b- In contrast to BCR, TCR has 2 antigen binding sites.
  - c- Both are cell surface molecules.
  - d- Both molecules cooperate in innate immune mechanisms.
  - e- They bind to antigens non-specifically.

## INNATE IMMUNITY

### **ILOs:**

**By the end of this chapter the student should be able to:**

- Describe mechanisms of innate immunity
- Describe the innate cellular defence mechanisms
- Define phagocytosis
- Define opsonization
- Describe different steps of phagocytosis
- Describe methods of killing in phagocytosis
- Describe inflammation

Any individual is protected from potentially harmful microorganisms in the environment by a number of very effective mechanisms present since birth. They do not depend upon prior exposure to any particular microorganism. They are non-specific and can act against any microorganism or foreign invader.

### **Mechanisms of Innate Immunity**

#### **I. Mechanical barriers and surface secretions**

1. Intact skin and mucous membranes constitute a barrier that cannot be penetrated by most microorganisms.
2. The sticky mucus covering mucous membranes traps any foreign material.
3. Cilia of the respiratory tract epithelium sweep foreign material out.
4. Blinking, sneezing and coughing reflexes expel foreign particles.
5. The flushing action of saliva, tears and urine helps in washing microbes from the body.
6. Sweat and sebaceous secretions contain substances (e.g. lactic acid and ammonia) that inhibit microorganisms.
7. Saliva, tears and mucous secretions of respiratory, alimentary and genitourinary tracts contain lysozyme which is bactericidal.
8. Gastric and vaginal acidity inhibit growth of microorganisms.

#### **II. Normal bacterial flora**

1. Bacteria of the normal flora produce bacteriocins and acids that destroy microorganisms.
2. They compete with pathogens for essential nutrients.

**N.B.:** Suppression of normal flora by antibiotics may lead to infection with potential pathogens (superinfection).

### III. Soluble defence factors

A number of microbicidal substances are present in tissues and body fluids and act in defence against microbes. They include:

1. **Lysozyme:** It is an enzyme that lyses bacteria by destroying the peptidoglycan of their cell wall.
2. **Complement:** It is a group of plasma proteins that act together to attack extracellular pathogens. The complement components are present in an inactive form, and can be activated either spontaneously by certain pathogens (where it is considered to be part of innate immunity) or by antibody binding to the pathogen (where it is considered to be part of acquired immunity) (Chapter 17).
3. **Acute phase proteins:** These are present at very low levels in normal serum, but their concentration rises dramatically, shortly after the onset of an infection. Microbial products, e.g. endotoxins, stimulate macrophages to release cytokines, which stimulate the liver to produce a large number of acute phase proteins. These proteins limit the spread of the infectious agent or stimulate the host response. Examples include fibrinogen and C-reactive protein (CRP).
4. **Interferons:** There are 2 types of interferons. Type I interferon ( $\alpha$  and  $\beta$ ) is part of innate immunity. It is secreted by virus-infected cells and prevents viral replication in uninfected neighbouring cells (i.e. it 'interferes' with viral replication).  
**N.B.:** Type II ( $\gamma$ ) Interferon is secreted mainly by T cells, and is considered part of the acquired immune response.

### IV. Cellular defence factors

#### 1. Phagocytes

Particles, e.g. bacteria, entering the tissue fluids or blood are rapidly engulfed by phagocytic cells. This process of engulfment (internalization) of particulate matter is termed phagocytosis. Phagocytes contain digestive enzymes capable of degrading ingested material.

The main phagocytic cells are:

- Neutrophils
- Monocytes/macrophages (monocytes in the blood and macrophages in the tissues)
- Dendritic cells

Table (3) shows a comparison between monocytes/macrophages and neutrophils.

**Phagocytosis** (Fig. 2): The process of phagocytosis occurs in subsequent steps:

- a- **Migration (Chemotaxis):** Microorganisms and injured tissues elaborate chemotactic factors that attract phagocytic cells to the site of infection. Some of the complement components and some cytokines may have chemotactic properties.



- b- **Attachment:** Phagocytes have receptors on their surface that can recognize non-specific molecules common to many pathogens, allowing attachment to them. Some microorganisms may alter these molecules or cover themselves with a thick capsule that is not recognized by any phagocyte receptor. Attachment may still occur if these microorganisms become coated with molecules which the phagocytes can recognize. These may be an antibody, a complement component or other molecules (e.g. CRP). In this case, the process is called **opsonization** and the substance which helped phagocytosis is called an opsonin.
- c- **Engulfment:** The cytoplasmic membrane of the phagocyte surrounds the organism and encloses it in a vacuole termed phagosome. Lysosomes, which are bags of enzymes, then fuse with the phagosome forming phagolysosomes, in which the engulfed material is killed and digested.
- d- **Killing:** This occurs by 2 mechanisms:
- **O<sub>2</sub> dependent mechanism:** After engulfment, there is a respiratory burst consisting of a steep rise in O<sub>2</sub> consumption. This is accompanied by an increase in the activity of a number of enzymes and leads to the generation of various reactive oxygen intermediates, such as hydrogen peroxide and singlet oxygen, which are lethal to microorganisms.
  - **O<sub>2</sub> independent mechanism:** These are the lysosomal enzymes (lysozyme, elastase, hydrolase ... etc.).

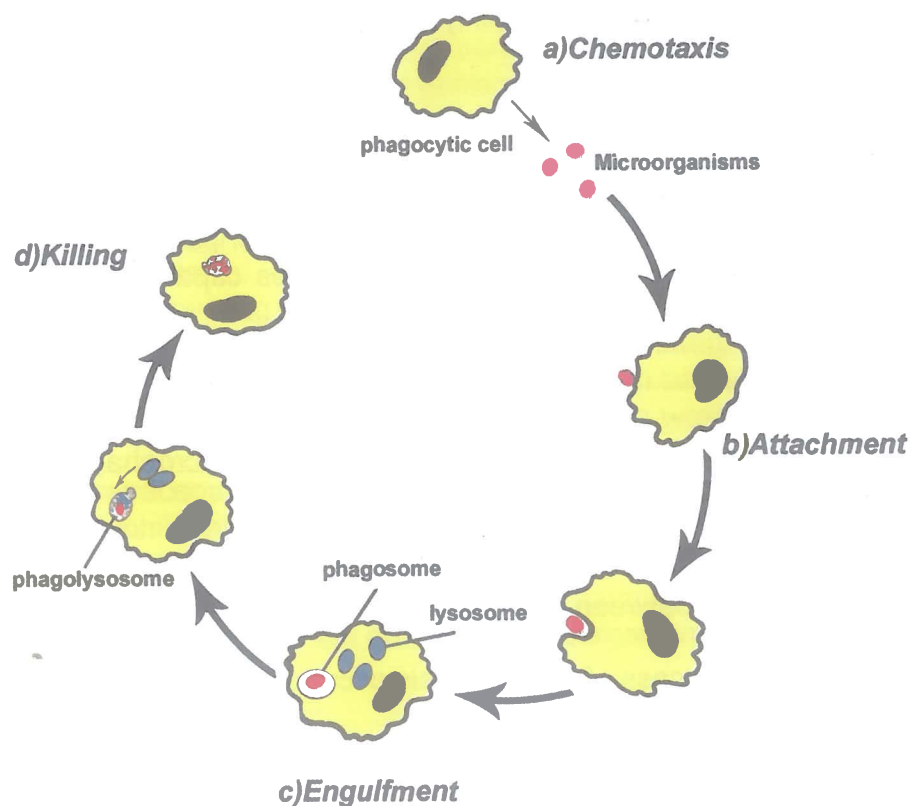


Fig. (2): Stages of phagocytosis

**N.B.:** In addition to phagocytosis, monocytes and macrophages have the following functions:

- **Antigen presentation:** Macrophages help to 'show' or 'present' part of the foreign agents they have eaten to T cells, so that the T cells can start responding to them. Thus, they are among a group of cells called **antigen presenting cells (APCs)**.
- **Secretion:** They secrete chemical mediators called **cytokines**, e.g. interleukins.
- **Direct cytotoxicity:** They may kill targets without engulfing them. Helminthic parasites which are too large to be engulfed can be killed by macrophages releasing their toxic contents onto them. Tumour cells can also be killed in a similar way.

**Table (3)** Comparison between neutrophils and monocytes/ macrophages

	<b>Neutrophils</b>	<b>Monocytes/ Macrophages</b>
<b>Importance</b>	They are the most numerous and most important cells of the innate immune system	In addition to phagocytosis they have other important functions
<b>Count</b>	<ul style="list-style-type: none"> <li>- 60 – 80% of TLC*</li> <li>- They are absent from normal tissues. During infections certain chemicals (chemotactic factors) are released to attract neutrophils from the blood to the site of infection.</li> </ul>	<ul style="list-style-type: none"> <li>- Monocytes form 1 – 5% of TLC</li> <li>- They continuously leave the blood to the tissues where they mature into macrophages. Examples: <ul style="list-style-type: none"> <li>• The Kupffer cells of the liver</li> <li>• The alveolar macrophages in the lung.</li> </ul> </li> </ul>
<b>Response during inflammation</b>	<ul style="list-style-type: none"> <li>- Rapid increase in production</li> <li>- Rapidly form pus</li> </ul>	<ul style="list-style-type: none"> <li>- Slight increase in blood levels</li> <li>- Slowly form granuloma</li> </ul>
<b>Size</b>	Small	Large
<b>Life-span</b>	Short: Die after phagocytosis, forming pus cells	Long: Survive after phagocytosis
<b>Functions</b>	Phagocytosis	<ol style="list-style-type: none"> <li>1. Phagocytosis</li> <li>2. Antigen presentation</li> <li>3. Cytokine secretion</li> <li>4. Direct cytotoxicity</li> </ol>

\*TLC: Total leucocytic count

## 2. Eosinophils

- They are granulocytes present mainly in tissues.
- **Count:** In the blood, they form 1-3% of TLC.

- **Functions:**

1. They are mainly of importance in **defence against helminthic parasite infections**. Such parasites, which are too large to be phagocytosed, can be killed by eosinophils releasing the toxic contents of their granules onto them.
2. They also play an important role in **allergic reactions**.
3. Eosinophils also have phagocytic properties.

### 3. Basophils and mast cells:

- **Basophils** are found in the blood in very low concentrations (0-2% of TLC).
- **Mast cells** are their tissue resident form. They are present either around the blood vessels or in the submucosa.
- **Function:** Both basophils and mast cells have similar functions. They possess granules containing a number of important mediators such as histamine. Release of these mediators:
  - Contributes to inflammation
  - Plays an important role in allergy

### 4. Natural killer cells

- They are large granular lymphocytes which can be distinguished from B and T lymphocytes.
- **Count:** They constitute 10-15% of peripheral blood lymphocytes.
- **Functions:**
  1. They are capable of non-specific killing of tumour cells and virus-infected cells in a manner similar to cytotoxic T cells; however, they differ from cytotoxic T cells in the way they recognize their targets.
  2. They secrete cytokines such as interferon  $\gamma$ .

## V. Inflammation

Chemical mediators released at the site of infection trigger an inflammatory response. The events that occur during inflammation are vasodilatation, increased vascular permeability and migration of leucocytes from the blood stream across the vascular endothelium into the inflamed tissues to combat the invading microbe. This migration is mediated by certain molecules, termed **adhesion molecules**, which are expressed on the surface of leucocytes and vascular endothelium.

**N.B.:** There is a lot of cooperation between innate and acquired immunity.

#### Examples:

- Innate immunity (macrophage) helps acquired immunity (T cell) through antigen presentation.
- Acquired immunity (antibody) helps innate immunity (macrophage) through opsonization.



**MCQS:**

- 1- All of the following is true regarding neutrophils **EXCEPT**:
  - a- They are phagocytic cells.
  - b- They constitute the majority of peripheral blood leucocytes.
  - c- They act mainly as part of the innate immune mechanism.
  - d- They are capable of killing abnormal cells by induction of apoptosis.
  - e- They are attracted to the site of infection by the action of chemotactic factors.
- 2- Macrophages perform the following functions **EXCEPT**:
  - a- Antigen presentation
  - b- Phagocytosis
  - c- Secretion of cytokines
  - d- Production of antibodies
  - e- Direct cytotoxicity
- 3- Which statement is **TRUE** concerning acute phase proteins?
  - a. They disappear from the circulation after onset of infection.
  - b. Endotoxins may stimulate their production.
  - c. They deprive pathogens from their essential nutrients.
  - d. They are produced by the primary lymphoid organs.
  - e. Type I IFN is an example of acute phase proteins.
- 4- All the following statements regarding phagocytosis are true **EXCEPT**:
  - a- Bacteria are engulfed in phagosomes.
  - b- Natural killer cells are the most important phagocytic cells.
  - c- Respiratory burst is lethal to microorganisms.
  - d- Antibodies may aid in recognition.
  - e- Complement components (C3b) enhance phagocytosis.
- 5- Enhanced phagocytosis is known as:
  - a- Agglutination
  - b- Opsonization
  - c- Neutralization
  - d- Antibody-dependent cellular cytotoxicity
  - e- Complement activation

## ANTIGENS

### ILOs:

By the end of this chapter the student should be able to:

- Define antigen and immunogen
- Define antigenic determinants or epitopes
- Define hapten
- Describe factors affecting immunogenicity

An **immunogen** is a substance that can stimulate the immune system to produce a specific immune response (humoral and/or cell-mediated) and can react specifically with the product of this response.

An **antigen**, on the other hand, may or may not be able to stimulate the immune system, but it can still specifically react with the product of the immune response.

Accordingly, all immunogens are antigens, but not all antigens are immunogens; however, the terms immunogen and antigen are incorrectly used interchangeably.

### Antigenic Determinants or Epitopes

- The immune system does not recognize the antigen molecule as a whole but reacts to limited parts of the molecule called epitopes.
- They are very small, composed of just four to five amino acids or monosaccharide residues.
- They determine the specificity of the antigen.
- The same antigen may possess different epitopes.
- Antigens that share one or more epitopes are known as cross-reactive (heterophil) antigens (Fig. 3).

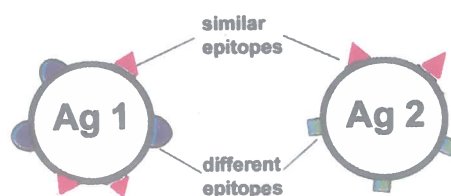


Fig. (3): Heterophil antigens

### Hapten

This is a low molecular weight substance which is incapable of inducing an immune response alone, but when coupled with a carrier molecule (protein) it can act as an immunogen. Examples of haptens are drugs e.g. penicillin.

## Factors Affecting Immunogenicity

### 1. Foreignness

For a molecule to be antigenic, it must be foreign to the host in which it is introduced. The immune system can normally distinguish between body components (self) and foreign substances (non-self) and is normally tolerant (non-reactive) to self-antigens (autotolerance).

### 2. Molecular Size

Usually, the larger the molecule, the stronger the antigenicity. However, there are exceptions; e.g. insulin is a small molecule but is immunogenic and carbon particles are very large but are non immunogenic.

### 3. Chemical Nature

The chemical complexity of a molecule contributes significantly to its immunogenicity. The more complex the molecule, the more immunogenic it is. The most potent immunogens are proteins.

### 4. Route of Administration

The route of administration of an antigen may affect the type and intensity of the immune response. As a general rule, the subcutaneous and intramuscular routes are the best in provoking an immune response.

### 5. Dosage

There is an optimum dose at which any antigen is most immunogenic. Very low or very high doses may result in a state of unresponsiveness (tolerance).

### 6. Adjuvants

Adjuvants are non-specific potentiators of the immune response. The administration of an adjuvant together with an antigen enhances the immune response to that antigen.

One of the commonly used adjuvants is aluminium hydroxide which is added to diphtheria and tetanus toxoids that are used for human immunization.

## MCQs:

- 1- All of the following statements about haptens are correct **EXCEPT**:
  - a. They are low molecular weight substances.
  - b. They are incapable of inducing an immune response alone.
  - c. They are non-specific potentiators of the immune response.
  - d. When coupled with a carrier molecule, they can act as antigens.
  - e. Penicillin is an example of a hapten.
- 2- Antigenicity is increased by all of the following **EXCEPT**:
  - a- Chemical complexity of antigen
  - b- Foreignness
  - c- Being protein in nature
  - d- Very high dose of antigen
  - e- Large size of antigen



## T CELL-MEDIATED IMMUNITY

### ILOs:

By the end of this chapter the student should be able to:

- List and describe different T cell surface molecules
- List and describe "professional antigen presenting cells"
- Recognize the two kinds of MHC molecules
- Explain how an antigen is presented to T lymphocytes
- Compare between cytosolic and vesicular pathogens
- Explain the MHC restriction theory
- Compare and contrast the presentation of exogenous and endogenous antigens to T lymphocytes
- Discuss the sequence of events in activation of naïve T cells
- Compare between naïve and effector T cells
- Recognize differences in function of T cell subtypes
- Discuss activation of macrophages by Th1 cells
- Discuss mechanism of killing by Tc cells
- Differentiate between NK cells and Tc cells
- Define the term: superantigens
- Differentiate between ordinary antigens and superantigens

The main functions of T cells are the destruction of intracellular pathogens as well as helping other cells of the immune system. For T cells to start functioning in the acquired immune response, they must first change from **naïve** T cells into **effector** T cells. Naïve T cells are activated to proliferate and differentiate into effector T cells the first time they recognize their specific antigen on the surface of an **antigen presenting cell (APC)**. T cells recognize antigens by their receptors (T cell receptors) with the help of a number of other molecules present on the T cell. They can only recognize antigen when it is carried on special molecules called **major histocompatibility (MHC) molecules**, present on the surface of the APCs. Understanding T cell activation and function, requires understanding of the most important T cell surface molecules as well as the APCs and the MHC molecules.

### T Cell Surface Molecules: (Fig. 4)

In addition to their role in antigen recognition and in interaction with other cells, some of these molecules serve as **markers** which help in identifying T cells and dividing them into subsets. The most important are:

**1. T Cell Receptor (TCR):** This receptor consists of two polypeptide chains called  $\alpha$  and  $\beta$  chains. In a manner similar to antibody molecules, both chains of TCR have a constant part near the cell membrane and a peripheral variable region. According to the structure of the variable region, the T cell receptor can recognize a certain antigen. All TCRs on a single T cell are identical and recognize the same antigen.

N.B.: A small proportion of TCRs are made of 2 different chains ( $\gamma$  and  $\delta$ ). T cells bearing such receptors differ from other T cells in recognition and effector mechanisms.

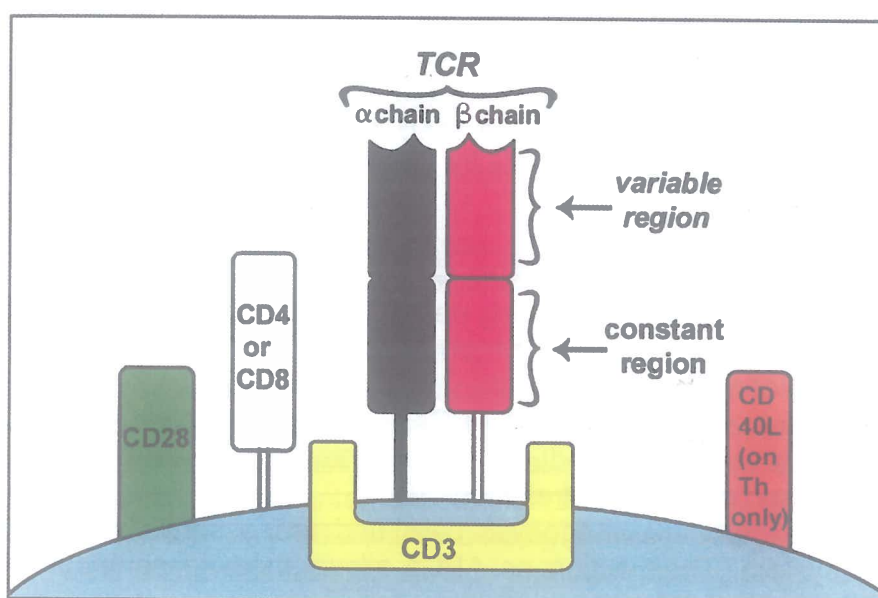


Fig. (4): T cell surface molecules

**2. CD3 Molecule:** This is found close to the TCR on all T cells and is involved in transmitting signals from the TCR to the inside of the cell.

**3. CD4 and CD8 Molecules:** T cells carry either CD4 or CD8 molecules on their surface and are thus classified into two major classes:

- CD4 T cells are called **helper T (Th) cells** and their main function is to help other cells of the immune system by secreting cytokines.

A small proportion of CD4 T cells are called regulatory T cells (Tregs); they inhibit immune responses.

- CD8 T cells are called **cytotoxic T (Tc) cells** and their main function is to kill infected cells, tumour cells and other cells carrying on their surfaces antigens which the T cell recognizes.

A small proportion of CD8 T cells are called suppressor T cells (Ts); they inhibit immune responses.

CD4 and CD8 molecules are also associated with the TCR during antigen recognition and are, therefore, called **co-receptors**.

The signal delivered through the TCR, with the help of the above-mentioned molecules, is considered the **first signal** needed for T cell activation.

**4. CD28 Molecule:** This molecule is present on all T cells. During the process of antigen recognition, it binds to a molecule called B7 present on the APC. This binding delivers a **second signal** necessary for T cell activation. CD28 is, therefore, called a **co-stimulatory molecule**.

**5. CD40 Ligand (CD40L):** This molecule is present on activated T helper cells and is involved in activation of B cells and macrophages by T cells. It binds to a molecule on B cells and macrophages called CD40 (hence the name CD40L).

**6. Fas Ligand (FasL):** This molecule is present on activated cytotoxic T cells and binds to **Fas** molecule present on various body cells. Interaction between both molecules occurs during killing of target cells by cytotoxic T cells.

### The Professional Antigen Presenting Cells

These are the only cells capable of activating naïve T cells. They are concentrated in the peripheral lymphoid tissues, such as lymph nodes, where they trap antigen and present it to the recirculating T cells:

- 1. Dendritic cells** are the most important APCs. They are so-called because they have cytoplasmic projections called dendrites. They are present in nearly all tissues of the body and are the most efficient APCs.
- 2. Macrophages** are important phagocytic cells and are essentially cells of the innate immune system. Their action as APCs allows them to contribute to the acquired immune response.
- 3. B cells** are mainly known for their action in humoral immunity, but they can also act as APCs.

### MHC Molecules

- The major histocompatibility complex (MHC) is the name given to a group of genes which code for the production of certain cell-surface glycoproteins called MHC molecules. Another name for them is the human leucocyte antigens (HLA antigens) because they were first discovered on the leucocytes. In addition to their role in antigen presentation, they have other significant roles which will be dealt with later (Chapter 12).
- There are two classes of MHC molecules which are important in T cell activation. They are called class I and class II MHC molecules (MHC I and MHC II). All people have both classes of MHC molecules.
- All nucleated cells of the body have MHC I molecules on their surfaces. The professional APCs have MHC II molecules, in addition to the MHC I molecules, on their surfaces.



## Antigen Presentation to T Cells

For antigens to be presented to T cells, they must first enter inside the APC, undergo degradation (antigen processing) producing peptides which are carried inside clefts on the MHC molecules to the cell surface, to be presented to T cells (**antigen presentation**).

The decision of whether antigenic peptides will be presented by MHC I or MHC II molecules depends upon the part of the cell in which the pathogen is present. This will decide the type of T cell activated and the resulting immune response.

There are two compartments in our cells in which infectious agents or their products can exist: the **cytosol** and the **vesicular system** (table 4):

**1. The Cytosol:** This is the fluid cytoplasm. All viruses and few bacteria live and replicate in this compartment. Antigens from pathogens in this compartment are sometimes called '**endogenous**' antigens.

**2. The Vesicular System:** This includes the phagosomes, lysosomes and other intracellular vesicles:

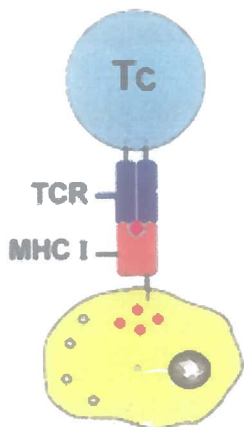
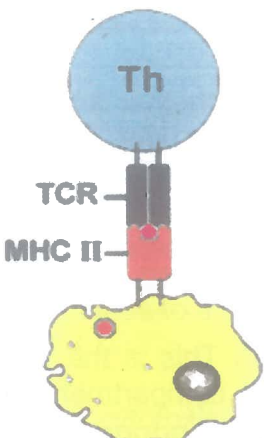
- Some very important intracellular bacteria (such as that causing tuberculosis), as well as some parasites, are engulfed by **macrophages**, and can live and replicate in the phagosomes inside them.
- Other bacteria which normally live extracellularly secrete toxins and other proteins. Such bacteria or their products can also be internalized by cells, thus reaching the vesicular system of the cell. Some viral components are also secreted extracellularly and can be internalized into the vesicular system in a similar manner. **B cells** are very efficient at binding extracellular pathogens and their products by the antibodies on their surface and internalizing them. **Dendritic cells** are also capable of harbouring antigens in their vesicular system.

Antigens from pathogens in the vesicular system are sometimes called '**exogenous**' antigens.

### Important Rules:

- Peptides arising from the **cytosol** are carried to the surface of the cell on **MHC I** molecules and can only be presented to **CD8 (cytotoxic) T cells**.
- Peptides arising from the **vesicular system** are carried to the surface of the cell on **MHC II** molecules and can only be presented to **CD4 (helper) T cells**.

**Table (4):** Comparison between cytosolic and vesicular pathogens

	Cytosolic "endogenous"	Vesicular "exogenous"
		
<b>Examples</b>	All viruses, few bacteria	<ul style="list-style-type: none"> <li>- Intracellular bacteria</li> <li>- Extracellular bacteria and their products when internalized</li> </ul>
<b>Degraded in</b>	Cytoplasm	Vesicles
<b>Peptides bind to</b>	MHC I molecules	MHC II molecules
<b>Presented to</b>	CD8 T cells	CD4 T cells
<b>Result</b>	Cytotoxic killing of presenting cell by CD8 T cell	Secretion of cytokines by CD4 T cells, giving help to macrophages, B cells and others

### MHC Restriction

This means that antigen recognition by T cells is restricted by the MHC molecules. This is true on two levels:

1. Since CD8 T cells recognize peptides bound to MHC I molecules and CD4 T cells recognize peptides bound to MHC II molecules, it is said that **CD8 T cells are "MHC I restricted"**, and **CD4 T cells are "MHC II restricted"**.
2. There is a variety of different possible shapes of MHC I and MHC II molecules (**MHC polymorphism**). Thus, one cell may have many different MHC I and MHC II molecules, and there are even more differences between MHC molecules on cells of different people.

The TCR is actually specific to the whole MHC-peptide complex, and not just the peptide alone. This means that a given T cell will recognize a certain peptide only when it is bound to a certain MHC molecule and will not recognize the same peptide bound to a different MHC molecule (Fig. 5).

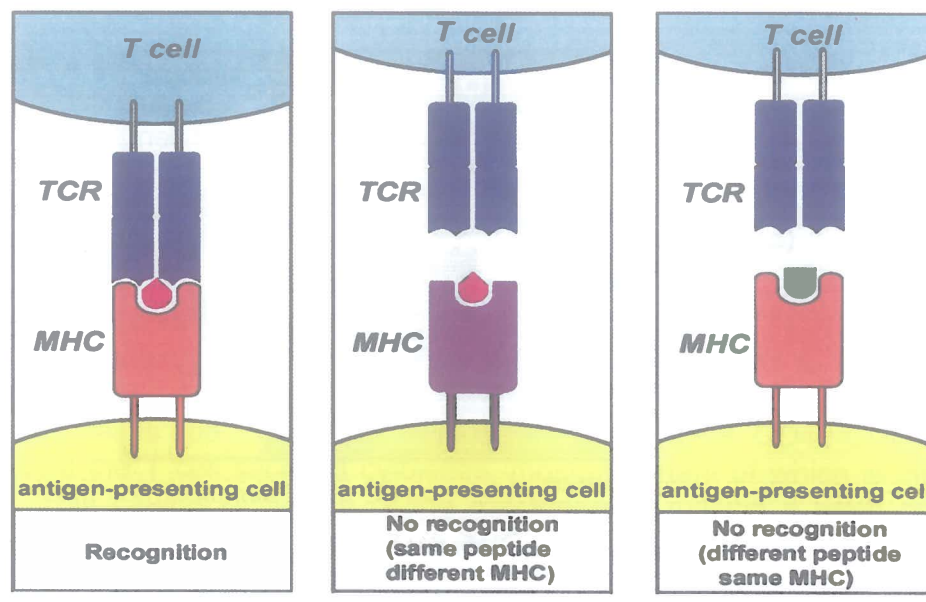


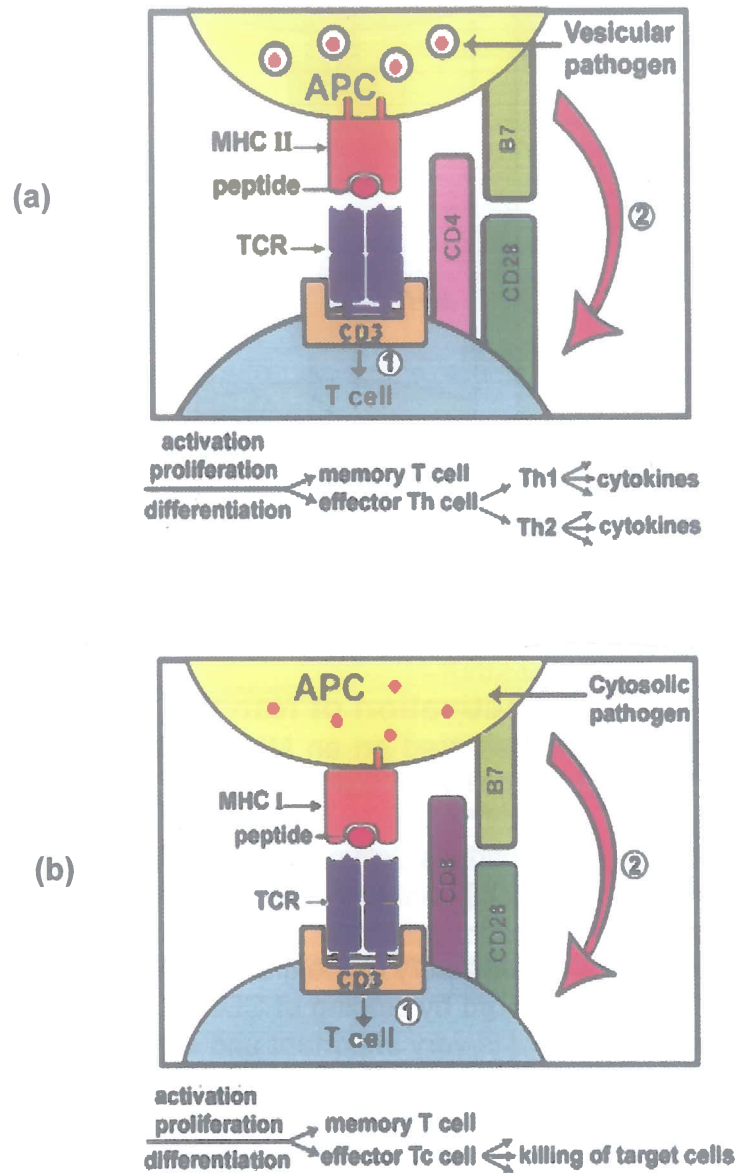
Fig. (5): MHC restriction

### Sequence of Events in Activation of Naïve T Cells: (Fig. 6)

- Any cytosolic peptide is delivered on an MHC I molecule to the surface of the APC. A CD8 (cytotoxic) T cell with TCRs specific for that peptide-MHC complex binds to it.
- Similarly, any vesicular peptide is delivered on an MHC II molecule to the surface of the APC. A CD4 (helper) T cell with receptors specific for that peptide-MHC complex binds to it.
- In either case, this leads to delivery of the first signal required for T cell activation.
- The second signal is delivered by binding of CD28 on the T cell to B7 on the APC (co-stimulation). This signal is very important and without it the T cell 'shuts down' and becomes non-responsive, a state called **anergy**.
- During these events, certain molecules, called **adhesion molecules**, present on the T cell and APC help to hold the two cells together.
- The T cell becomes a lymphoblast (activation), divides repeatedly (proliferation or clonal expansion) and its progeny (daughters) finally become effector cells (differentiation). Proliferation and differentiation are helped by interleukin-2 (IL-2), a cytokine secreted by the T cell itself.

This sequence of events is known as the **primary immune response**. In addition to the production of effector T cells, it also results in the development of **memory T cells**, which are long-lived T cells. Upon subsequent challenge with the same pathogen these memory T cells rapidly change into effector cells and provide quicker and more efficient protection (**secondary immune response**).





**Fig. (6): Activation of naïve T cells:**  
**(a) Activation of Th cells, (b) Activation of Tc cells**

- **Effector T cells differ from naïve T cells in the following:**
  - a. They are now ready to start performing their functions.
  - b. They can be triggered to act as soon as their TCRs bind their specific MHC-peptide complex (signal 1) without need for co-stimulation (signal 2).

## Functions of Effector T Cells

### I. Function of effector CD4 T cells (helper T cells):

The main function of effector CD4 T cells is to secrete cytokines, and according to the panel of cytokines they secrete, they are classified either as Th1 or Th2 cells:

**1. Th1 cells:** produce cytokines which predominantly activate macrophages and promote inflammation.

#### Mechanism of activation of macrophages by Th1 cells: (Fig. 7)

- An infected macrophage displays a peptide-MHC II complex to an effector Th1 cell.
  - If the Th1 cell is specific for that peptide-MHC II complex, CD40-CD40L binding occurs and the T cell secretes cytokines, most important of which is interferon- $\gamma$  (IFN- $\gamma$ ). This causes activation of the macrophage, making it more capable of killing the bacteria it harbours:
    - There is more efficient fusion between the phagosomes containing the bacteria and the lysosomes containing the bactericidal enzymes.
    - There is also increased production of oxygen radicals, nitric oxide and antibacterial enzymes by the macrophage.
- All this leads to effective killing of the intracellular bacteria.

Activation of Th1 cells and consequent macrophage activation can sometimes cause significant tissue damage. However, its absence can lead to serious consequences of disseminated infection. This is typically seen in AIDS patients with mycobacterial infection, since the number of Th cells in these patients is very low.

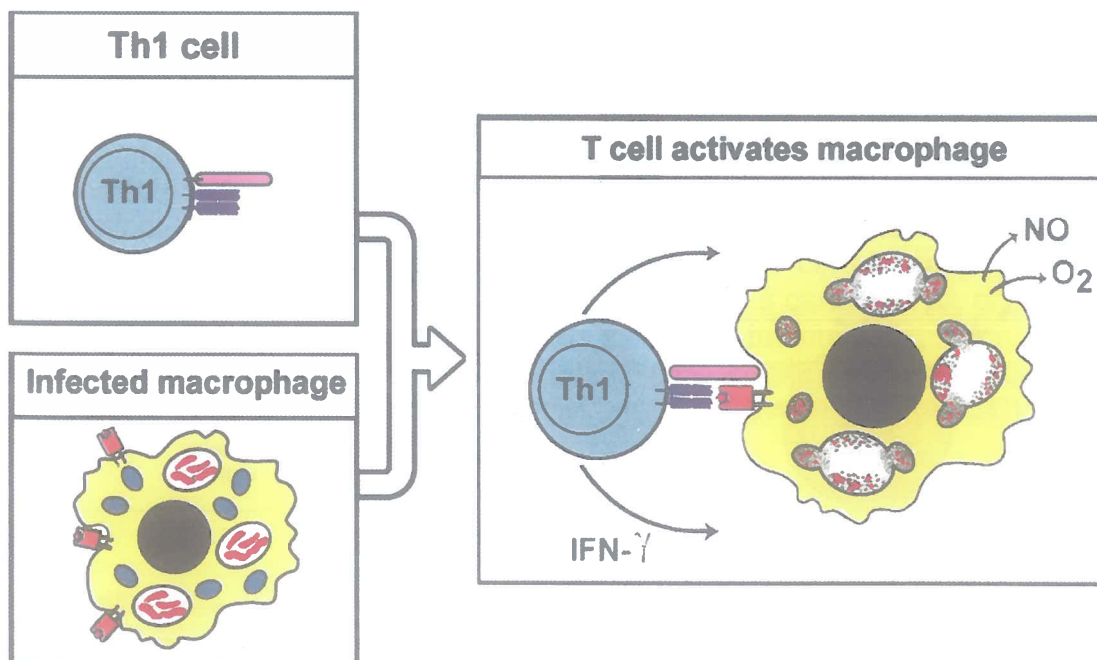


Fig. (7): Mechanism of activation of macrophages by Th1 cells

**2. Th2 cells:** produce cytokines which predominantly help B cells and promote humoral immunity. Details of this are discussed in the chapter on humoral immunity (Chapter 6).

Details of the cytokines produced by Th1 and Th2 cells, and a comparison between both groups of cells are present in Chapter 5.

## II. Function of Effector CD8 T Cells (Cytotoxic T Cells):

The main function of effector CD8 T cells is to eliminate abnormal cells, such as virus-infected cells or tumour cells, which could be dangerous to the body as a whole.

- The target cell, such as a virus-infected cell, displays on its surface viral peptide in conjunction with an MHC I molecule.
- An effector Tc cell with TCR specific for that peptide-MHC complex recognizes it and delivers a lethal hit, leading to death of the target cell.
- After inducing death of the target cell, the Tc cell detaches and searches for other similar target cells to kill.

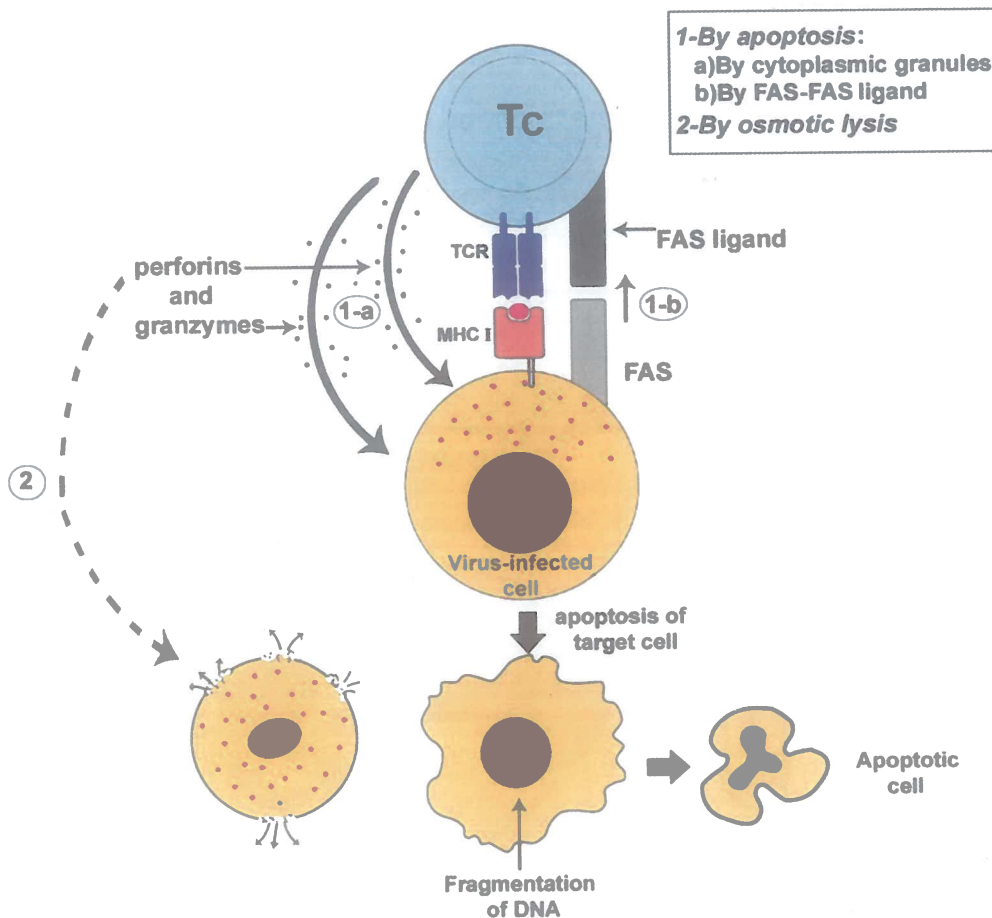


Fig. (8): Mechanism of killing by Tc cells



**Mechanism of killing by Tc cells: (Fig. 8)**

Two mechanisms are involved:

**1. Induction of apoptosis:** The Tc cell induces the target cell to undergo suicide. This process of suicide is called **apoptosis**. It may occur by one of two mechanisms:

**a- Release of cytoplasmic granules:** The cytotoxic cell releases two kinds of granules called **perforins** and **granzymes**. The perforins create perforations (holes) in the cytoplasmic membrane of the target cell. Through these holes, granzymes enter the target cell and cause activation of enzymes naturally present in it, leading to degradation of the cell's DNA.

**b- Interaction of cell surface molecules:** A receptor called **FAS** (factor for apoptotic signal) present normally on many cells, binds to a molecule called **FAS-ligand**, present on cytotoxic cells, giving a signal for apoptosis in the target cell.

Apoptosis is a clean death in which the cell destroys itself from within, shrinking and degrading itself until little is left. The enzymes which are activated to destroy cellular DNA can also degrade nucleic acid of intracellular pathogens, thus preventing spread of infection.

**2. Osmotic lysis:** The pores produced in the target cell membrane by the perforins may allow entrance of fluid into the cell, leading to death of the target cell by osmotic lysis.

Death by apoptosis is faster than osmotic lysis and is probably the main mechanism involved in killing of target cells by Tc cells.

**N.B.: Natural Killer cells (NK cells)** are cells of the innate immune system which are capable of killing target cells using the same mechanisms as Tc cells. However, certain important differences exist between NK cells and Tc cells:

- 1- NK cells exhibit a natural ability to recognize and kill abnormal cells, such as virus-infected cells or tumour cells, without need for antigen-specific-MHC activation as required by T cells. Thus, they are important in the early stages of infection, before development of the acquired immune response.
- 2- NK cells do not show specificity to cells bearing a certain antigen. In other words, the same NK cell can kill a cell infected with a certain virus, and go on to kill another cell infected by another virus, or even a tumour cell.
- 3- Sometimes antibodies can help NK cells to recognize abnormal cells. This mechanism is called antibody-dependent cellular cytotoxicity (ADCC) (chapter 16).

**Significant points related to function of T cells:**

1. The lifestyle of the pathogen (where it lives and which compartment of the cell it enters) decides whether **cytotoxic** or **helper** T cells will be stimulated and consequently the *type of immune response*. The immune response in each case is **exactly what is needed** for defence against that pathogen:

- When the pathogen is in the **cytosol** (e.g. a virus), the only solution is to kill that cell by **cytotoxic** T cells, since it harbours numerous virus particles which may infect other cells. This shows the importance of the MHC I → CD8 connection.
  - When the pathogen is in the **vesicular** system, there is no indication for activation of cytotoxic cells and killing of cells. Vesicular pathogens are either intracellular pathogens living inside macrophages, or are products of extracellular pathogens which have been internalized by B cells. In these cases, activation of **helper** T cells is needed. Cytokines released by helper T cells will help in activation of macrophages and B cells. This shows the importance of the MHC II → CD4 connection.
2. The significance of the presence of **MHC I** on all nucleated cells of the body is clear. Any cell of the body is liable to be infected by a virus, so all cells must possess MHC I molecules in order to 'show' viral peptides to the Tc cell so that it can kill that infected cell; otherwise it would escape killing. In other words, the presence of MHC I molecules on all body cells is necessary so that these cells can be **targets** for the Tc cells if the need arises.
- The significance of the presence of **MHC II** on only a few cells of the immune system (mainly the professional APCs) is also clear. Presentation of antigen to Th cells results in production of cytokines. These cause activation of many cells of the immune system, with many consequences. There is no need for the presence of MHC II on all cells of the body; in fact this would lead to over-stimulation of Th cells and over-production of cytokines, which would be dangerous to the body.

### Superantigens: (Fig. 9)

- Certain proteins secreted by some pathogens do not act like ordinary antigens (Table 5). They are not processed and presented to T cells like ordinary antigens, but have the ability to bind directly to the MHC II molecule on the surface of the APC without entering the cell, and at the same time to the variable portion of the  $\beta$  chain of the TCR, acting as a clamp between both molecules.
- This type of binding to TCR is not very specific as in ordinary activation of T cells and, consequently, very large numbers of Th cells can be activated by one kind of superantigen. That is why they are called 'superantigens'.
- The result is release of huge amounts of cytokines, which is not beneficial to the host and even causes systemic toxicity. There is suppression of the normal acquired immune response and no memory cells are produced.
- Superantigens are produced by many different bacteria and viruses and are effective at very low concentrations. Well known examples of superantigens are staphylococcal **enterotoxins** and **toxic shock syndrome-toxin**. The overproduction of cytokines in response to these toxins accounts for many of their toxic effects on the body.

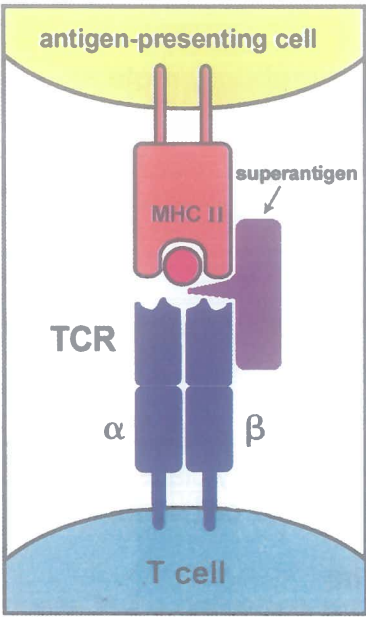


Fig. (9): Activation of T cells by superantigens

Table (5): Comparison between ordinary antigens and superantigens

	Ordinary Antigen	Superantigen
Processing inside APCs	Yes	No
Presentation by MHC molecules	Yes	No
Site of binding to MHC molecule	Peptide-binding cleft	Outside peptide-binding cleft
Binding to TCR	Variable portion of α and β chains	Variable portion of β chain
Specificity of TCR to it	Very specific	Not very specific
Acquired immune response	Stimulated	Suppressed
Development of memory	Yes	No
Result of T cell stimulation	Usually beneficial to host	Usually harmful to host

**Cell mediated immunity (CMI)** is a very important term related to T cell functions. It is applied to defensive reactions that are mediated primarily by activated T lymphocytes and macrophages. It is important in defence against intracellular pathogens, such as viruses, some bacteria and also against fungi. The two main effector functions of CMI are the cytotoxicity mediated by Tc cells and the macrophage activation and inflammation mediated by Th1 cells.



**MCQs:**

- 1- One of the following is a co-stimulatory molecule:
  - a- CD40
  - b- CD40L
  - c- CD28
  - d- CD3
  - e- TCR
- 2- Regarding helper T cells, all of the following statements are true **EXCEPT**:
  - a. They activate macrophages.
  - b. They activate B cells.
  - c. They lyse target cells.
  - d. They recognize antigen presented by class II MHC molecules.
  - e. They have CD4 molecules on their cell surface.
- 3- Dendritic cells are involved in:
  - a- Transporting the epitope to the surface of a B cell
  - b- Suppressing the immune system
  - c- Destroying target cells
  - d- Presenting peptides to naive T cells
  - e- Producing antibody
- 4- Extracellular bacteria are presented:
  - a- With MHC I to CD4 Th cells
  - b- With MHC I to CD8 Tc cells
  - c- With MHC II to B cells
  - d- With MHC II to CD4 Th cells
  - e- With MHC II to CD8 cells
- 5- The second signal needed for the activation of naïve T cells is delivered when:
  - a- CD40 ligand binds to CD40 molecule
  - b- CD4 binds to MHCII molecule
  - c- CD8 binds to MHCI molecule
  - d- CD3 binds to TCR
  - e- CD28 binds to B7 molecule
- 6- Which statement about superantigens is **INCORRECT**?
  - a- They bind directly to MHC II molecule outside the peptide binding cleft.
  - b- They result in the release of huge amount of cytokines.
  - c- They result in stimulation of the normal acquired immune response.
  - d- They bind to the variable portion of the  $\beta$  chain of the TCR.
  - e- Unlike ordinary antigens, they are not processed in APCs.
- 7- Cytotoxic T cells exert their killing by:
  - a. Antibodies with specific recognition capabilities
  - b. Inserting the complement components into target cell membrane
  - c. T cell antigen receptors and MHC protein
  - d. Inserting a pore-forming protein called perforin into target cell membrane
  - e. Activation of macrophages

## CYTOKINES

### **ILOs:**

**By the end of this chapter the student should be able to:**

- Define cytokines
- List general characteristics of cytokines
- Classify and discuss cytokines that mediate and regulate innate and specific immunity
- Identify the role of cytokines in the activation, growth and differentiation of lymphocytes
- Differentiate between Th1 and Th2 cells
- Describe cytokines that stimulate haematopoiesis
- Explain therapeutic uses of cytokines

Cytokines are peptide or glycoprotein mediators that are mainly produced by cells of the immune system and have an effect on the behaviour and properties of many cells. Although T cells are the major source of cytokines, many other cells can produce them, and they have a wide range of functions, extending beyond the immune system (e.g. wound healing).

Many different and overlapping names have been given to the various cytokines:

- Cytokines produced by lymphocytes are often called **lymphokines**
- Many cytokines are given the name **interleukin (IL)**, followed by a number (eg. IL-2).
- **Chemokines** are cytokines that are involved in the migration and activation of cells, especially phagocytic cells.
- **Interferons** are cytokines capable of inducing body cells to resist viral replication, but they have other important functions, as well.

## General Characteristics of Cytokines

1. They are highly potent, often acting at very low concentrations.
2. They act through high-affinity cell surface receptors.
3. Their action is transient.
4. They act mainly in an **autocrine** manner (affecting the cell which produced them) or in a **paracrine** manner (affecting cells close by).
5. They are **pleiotropic**, i.e. the same cytokine may have multiple effects.
6. Different cytokines may have the same activity (**redundancy**).
7. They may act sequentially (**network interaction**). They can also act together and increase the effect of one another (**synergy**), or act as **antagonists**.

More than 100 cytokines have been identified so far. Classifying cytokines is difficult because many are produced by more than one cell type and many have overlapping actions. Fig. (10) shows a schematic overview of the most important cytokines. They can be classified into three main groups:

### I. Cytokines that Mediate and Regulate Innate Immunity

These cytokines are produced mainly by cells of the innate immune system and their actions serve to mediate innate immunity. However, some of them are produced by other cells as well and their actions can also affect cells of the specific immune system. Tables (6A and 6B) summarize the most important cytokines of this group. Monocytes/macrophages produce all of these cytokines; some are produced by other cells, as well.

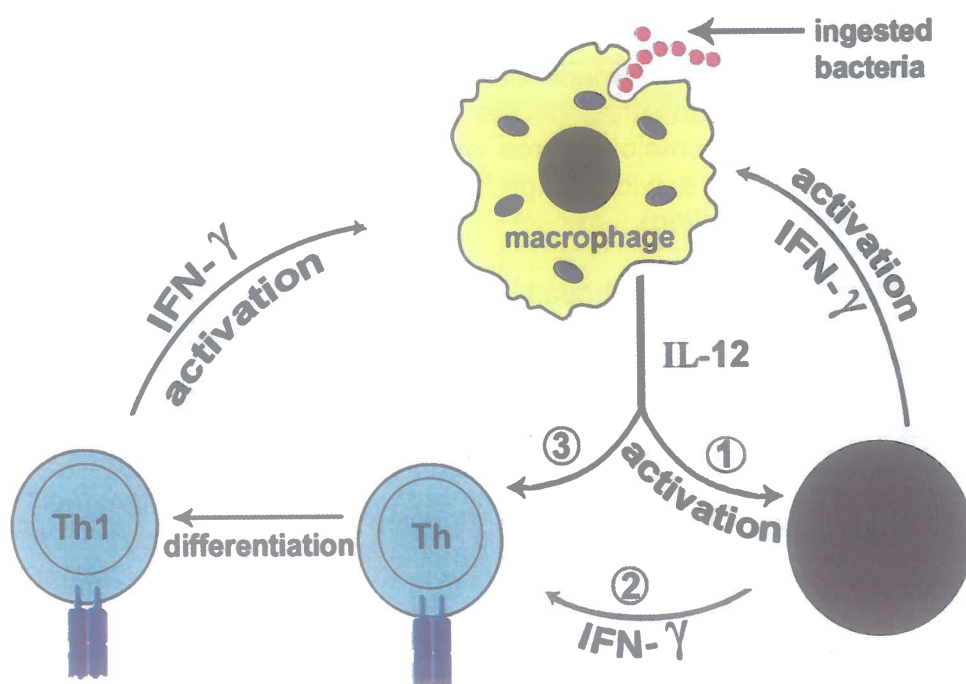


Fig. (11): Actions of IL-12



Fig. (10): Schematic overview of important cytokines.

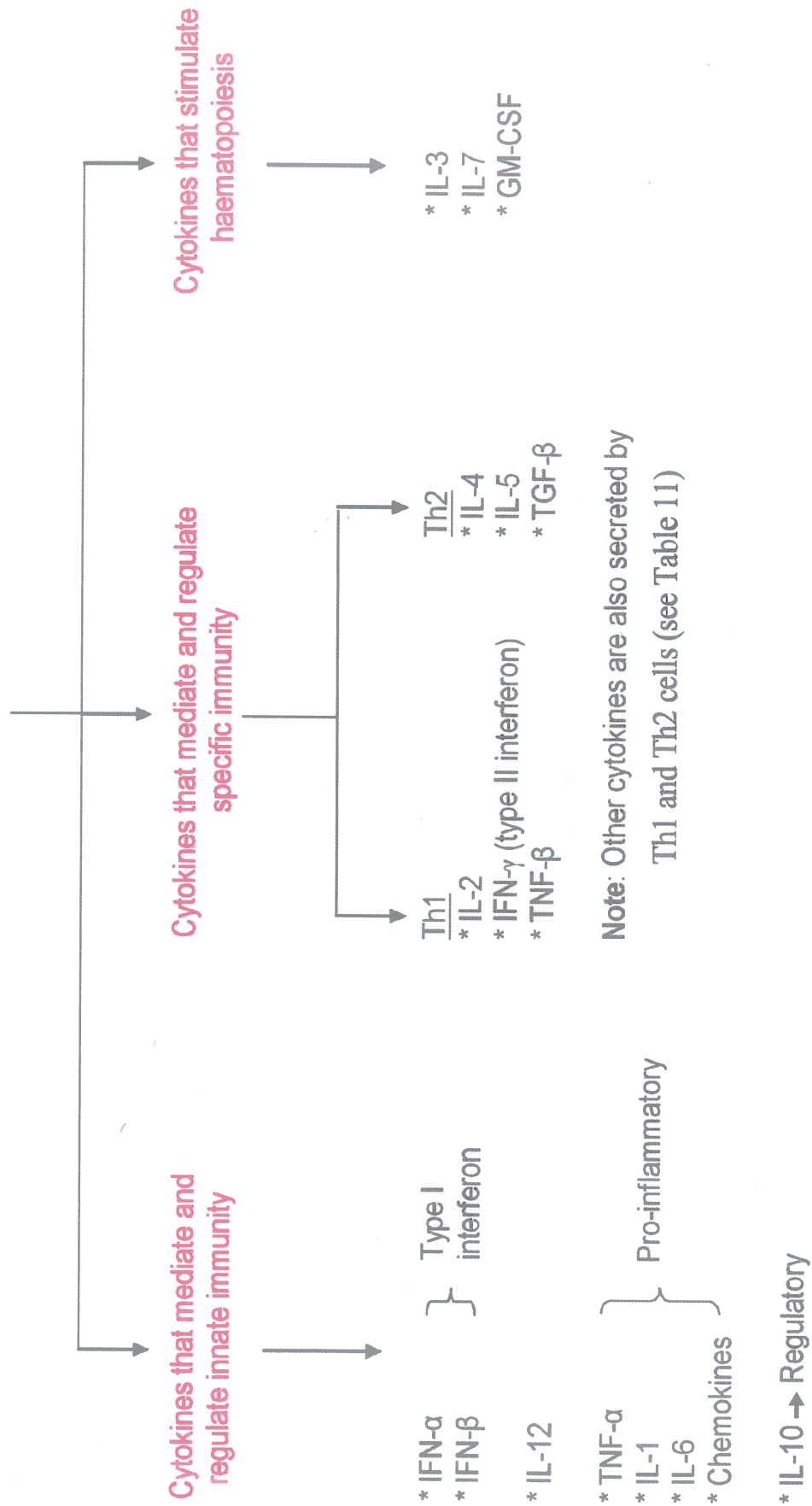


Table (6A): Cytokines that mediate and regulate innate immunity

	Type I Interferon (Type I IFN)	Interleukin-12 (IL-12)	Interleukin-10 (IL-10)
<b>Importance</b>	<ul style="list-style-type: none"> <li>• It is very important in viral infections.</li> <li>• It includes IFN-<math>\alpha</math> and IFN-<math>\beta</math>.</li> </ul>	<ul style="list-style-type: none"> <li>• Acts as an important link:               <ul style="list-style-type: none"> <li>- Between macrophages and NK cells</li> <li>- Between innate immunity and specific immunity</li> </ul> </li> <li>• Mainly monocytes/macrophages</li> </ul>	<ul style="list-style-type: none"> <li>• A regulatory cytokine</li> </ul>
<b>Source</b>	<ul style="list-style-type: none"> <li>• IFN-<math>\alpha</math> is produced mainly by monocytes/ macrophages.</li> <li>• IFN-<math>\beta</math> is produced mainly by fibroblasts.</li> <li>• Many other cells also produce both.</li> </ul>	<ul style="list-style-type: none"> <li>• Mainly monocytes/macrophages</li> </ul>	<ul style="list-style-type: none"> <li>• Macrophages</li> <li>• Th2 cells</li> <li>• Other cells</li> </ul>
<b>Stimulus</b>	<ul style="list-style-type: none"> <li>• Most important stimulus: viral infection</li> </ul>	Stimulation of monocytes/ macrophages by bacterial constituents	
<b>Actions</b>	<p>1. Inhibition of viral replication: Type I IFN causes cells to synthesize a number of enzymes that interfere with the translation of viral mRNA. This antiviral action is mainly paracrine, meaning that a virally-infected cell secretes IFN to protect neighbouring cells not yet infected. A cell that has responded to IFN is resistant to any viral infection and is said to be in an anti-viral state.</p> <p>2. Activation of NK cells: This is very important early in the course of infection, before the onset of the specific immune response.</p> <p>3. Increased expression of MHC I molecules: This leads to more recognition of viral peptides and more efficient killing of virally-infected cells by CD8 Tc cells.</p> <p>4. Inhibition of cell proliferation</p> <p><b>Note:</b> The first three actions of type I interferon act together to eradicate viral infections, while the fourth action is important against tumours.</p>	<p>1. It increases the cytotoxic activity of NK cells</p> <p>2. It stimulates NK cells to secrete IFN-<math>\gamma</math>.</p> <p>3. It promotes the differentiation of Th cells into Th1 cells. These, in turn, produce IFN-<math>\gamma</math> which activates macrophages.</p> <p>(The link between macrophages, NK cells and Th cells is shown in Fig. 11).</p>	Anti-inflammatory

(Table 6B): Cytokines that mediate and regulate innate immunity (Cont.)

Pro-inflammatory cytokines				
	<b>Tumour Necrosis Factor-<math>\alpha</math> (TNF-<math>\alpha</math>)</b>	<b>Interleukin-1 (IL-1)</b>	<b>Interleukin-6 (IL-6)</b>	<b>The chemokines</b>
<b>Importance</b>	It is the principal mediator of innate immune response against Gram-negative bacteria.	An important mediator in the host inflammatory response in innate immunity	Same as IL-1	These are chemotactic cytokines that are capable of stimulating leucocyte movement in a certain direction. <b>Example:</b> Interleukin-8
<b>Source</b>	<ul style="list-style-type: none"> <li>Mainly monocytes/macrophages</li> <li>Th1 cells</li> </ul>	Mainly monocytes/macrophages	<ul style="list-style-type: none"> <li>Monocytes/macrophages</li> <li>Th2 cells</li> <li>Others</li> </ul>	Monocytes/macrophages
<b>Stimulus</b>	<ul style="list-style-type: none"> <li>Stimulation of monocytes/macrophages by bacterial lipopolysaccharide (endotoxin).</li> </ul>		Infections and trauma	
<b>Actions</b>	<ol style="list-style-type: none"> <li>Small quantities of TNF-<math>\alpha</math> act locally to recruit and activate neutrophils and other cells to combat bacterial infections, especially those caused by Gram-negative bacteria.</li> <li>In large quantities, TNF-<math>\alpha</math> enters the blood stream, causing systemic effects such as fever, shock and even death. Many of the manifestations of septic shock are actually caused by over-secretion of TNF-<math>\alpha</math>.</li> <li>Pro-inflammatory actions: <ul style="list-style-type: none"> <li>Production of fever</li> <li>Promotion of local inflammation</li> <li>Induction of synthesis of acute phase proteins</li> </ul> </li> <li>It synergizes with IFN-<math>\gamma</math> in activation of macrophages (see later).</li> <li>It has cytotoxic activity against some cells, such as tumour cells.</li> </ol>	Same pro-inflammatory actions as TNF- $\alpha$	<ol style="list-style-type: none"> <li>Pro-inflammatory actions</li> <li>Growth factor for B cells</li> </ol>	Chemotactic for neutrophils



II. Cytokines that Mediate and Regulate Specific Immunity:

These cytokines are produced mainly by Th cells and mediate their actions. Some are produced by Th1 cells, some by Th2 cells and some by both (Table 7).

Table (7): Comparison between Th1 and Th2 cells:

	Th1	Th2
Cytokines produced	IL-2 IFN- $\gamma$ TNF- $\alpha$ and $\beta$  GM-CSF IL-3	IL-4 IL-5 IL-6 IL-10 TGF- $\beta$  GM-CSF IL-3
Development promoted by	IL-12 IFN- $\gamma$ Large doses of antigen	IL-4 Small doses of antigen
Development inhibited by	IL-4 IL-10	IFN- $\gamma$
Promote	Cell-mediated immunity	Humoral immunity

Tables (8A and 8B) summarize the most important cytokines produced by Th1 and Th2 cells respectively.

III. Cytokines that Stimulate Haematopoiesis

These are cytokines that support the production of all blood cells, or particular blood cells (Table 9).

Table (9): Cytokines that stimulate haematopoiesis

	Interleukin-3 (IL-3)	Granulocyte-monocyte colony stimulating factor (GM-CSF)	Interleukin-7 (IL-7)
Source	• Th1 cells • Th2 cells	• Th1cells • Th2 cells	Thymic and bone marrow stromal cells
Actions	It promotes formation of blood cells	It promotes development of granulocytes and monocytes.	It promotes production of T and B cells.

Table (8A): Cytokines produced by Th1 cells

	<b>Interleukin-2 (IL-2)</b>	<b>Interferon-<math>\gamma</math> (IFN-<math>\gamma</math>) (Type II Interferon) (Immune Interferon)</b>	<b>Tumour Necrosis Factor-<math>\beta</math> (TNF-<math>\beta</math>)</b>
<b>Importance</b>	It is mainly known as an autocrine and paracrine growth factor for T cells.	It is the hallmark of Th1 cells, i.e. production of IFN- $\gamma$ defines a Th cell as a Th1 cell.	
<b>Source</b>	Th1 cells	<ul style="list-style-type: none"> <li>• Mainly by Th1 cells</li> <li>• NK cells</li> </ul>	Th1 cells
<b>Actions</b>	<ol style="list-style-type: none"> <li>1. It promotes proliferation of T cells, and B cells.</li> <li>2. It promotes cytokine production by T cells.</li> <li>3. It activates NK cells so that their killing ability is enhanced. NK cells activated by IL-2 become <b>lymphokine-activated killer cells (LAK cells)</b>.</li> </ol>	<ol style="list-style-type: none"> <li>1. Activation of macrophages (It synergizes with TNF-<math>\alpha</math> in this action):               <ul style="list-style-type: none"> <li>- It promotes fusion of phagosomes containing the bacteria to lysosomes containing anti-bacterial substances.</li> <li>- It induces synthesis of nitric oxide and other bactericidal substances.</li> <li>- It induces macrophages to secrete their cytokines.</li> </ul> </li> <li>2. It increases the expression of MHC I molecules, leading to better killing of target cells by Tc cells.</li> <li>3. It increases the expression of MHC II molecules on APCs, leading to better presentation of antigens to Th cells.</li> <li>4. It promotes the development of Th1 cells and inhibits the development of Th2 cells.</li> <li>5. It activates NK cells.</li> </ol>	It has actions similar to TNF- $\alpha$ (see before).

Table (8B): Cytokines produced by Th2 cells

	<b>Interleukin-4 (IL-4)</b>	<b>Interleukin-5 (IL-5)</b>	<b>Transforming growth factor-<math>\beta</math> (TGF-<math>\beta</math>)</b>
<b>Importance</b>	<b>It is the hallmark of Th2 cells, i.e. production of IL-4 defines a Th cell as a Th2 cell.</b>	<b>It plays an important role in allergic diseases and control of helminthic infections.</b>	<b>An immunosuppressive cytokine</b>
<b>Source</b>	<ul style="list-style-type: none"><li>• Th2 cells</li><li>• NK cells</li><li>• Mast cells</li></ul>	Th2 cells.	<ul style="list-style-type: none"><li>• Th2 cells</li><li>• Macrophages</li></ul>
<b>Actions</b>	<ol style="list-style-type: none"><li>1. It helps activation and growth of B cells.</li><li>2. It promotes production of IgE.</li><li>3. It promotes growth and function of mast cells and eosinophils. (The above three actions promote the development of type I hypersensitivity).</li><li>4. It promotes the development of Th2 cells and inhibits the development of Th1 cells.</li><li>5. It suppresses the synthesis of the pro-inflammatory cytokines.</li></ol>	<ol style="list-style-type: none"><li>1. It promotes growth and differentiation of eosinophils.</li><li>2. It is a B cell growth factor.</li></ol>	<ol style="list-style-type: none"><li>1. It was originally discovered as a growth factor that promotes wound healing.</li><li>2. It promotes production of IgA.</li><li>3. It is an immunosuppressive cytokine.</li></ol>



## Therapeutic Uses of Cytokines

There is considerable interest in the possible use of cytokines as therapeutic agents, either to augment an immune response, or to inhibit inflammation (by using anti-inflammatory cytokines such as IL-10 and TGF- $\beta$ ). However, the administration of cytokines in therapeutic doses may lead to toxicity.

### Examples of Therapeutic Uses

#### Interferon- $\alpha$ (IFN- $\alpha$ )

- Has shown success in treatment of viral hepatitis.
- Is being tested as a possible treatment for many malignancies especially lymphomas and leukaemias.

#### Interleukin-2 (IL-2)

Experimentally, its administration to normal or immunodeficient mice enhances immune responses, but use in humans is limited by severe toxic side effects.

#### Granulocyte-monocyte colony stimulating factor (GM-CSF)

They can be used to treat cases of leucopenia and bone marrow depression.

### MCQs:

- 1- Cytokines are characterized by all the following **EXCEPT**:
  - a- They may have a direct effect on the cells producing them.
  - b- They may have antagonistic effects.
  - c- Those affecting migration of lymphocytes are called chemokines.
  - d- Their action is antigen-specific.
  - e- They attach to specific cell surface receptors.
- 2- One of the following is a pro-inflammatory cytokine:
  - a- IL-4
  - b- IL-5
  - c- TGF- $\beta$
  - d- IL-3
  - e- TNF- $\alpha$
- 3- Th1 cells are characterized by one of the following:
  - a- They produce IL-4.
  - b- They are activated by IL-4.
  - c- Their development is promoted by IL-4.
  - d- Their development is inhibited by IL-4.
  - e- Their functions are enhanced by IL-4.
- 4- All of the following statements about Th2 cells are true **EXCEPT**:
  - a. They produce IL-3, 4, 5 and 6.
  - b. Their development is inhibited by IFN- $\gamma$ .
  - c. They promote cell-mediated immunity.
  - d. Their development is promoted by IL-4.
  - e. They produce TGF- $\beta$ .

- 5- **IL-2 is produced by:**
- a- Activated macrophage
  - b- NK cells
  - c- Activated Th1
  - d- Th2
  - e- B lymphocytes
- 6- **Interferon gamma:**
- a- Is considered the hallmark of Th2 cells
  - b- Promotes haematopoiesis
  - c- Is produced by activated macrophages
  - d- Is also called type I interferon
  - e- Increases expression of MHC molecules on different cells
- 7- **One of the following cytokines promotes growth and differentiation of eosinophils:**
- a- IL-2
  - b- IL-10
  - c- TNF- $\alpha$
  - d- IL-5
  - e- IL-1

## THE HUMORAL IMMUNE RESPONSE

### **ILOs:**

**By the end of this chapter the student should be able to:**

- List receptors expressed on the surface of the B cells
- Explain sequence of events in activation of naïve B cells
- Define immunoglobulins
- Contrast differences between T and B cell receptors
- Summarize the role of T helper lymphocytes, in particular how they interact with B lymphocytes and macrophages
- Describe the mechanism of antigen-induced B lymphocyte activation
- Identify the role of cytokines in the activation, growth and differentiation of lymphocytes
- Compare and contrast the effects of T dependent & T independent antigens
- Correlate structure of immunoglobulin molecule with its function
- Summarize antibody structure and function
- Recall functions of different immunoglobulin isotypes
- Define hypervariable regions
- Discuss the significance of immunoglobulin class switching
- Compare between the primary and secondary antibody response
- Identify heterophil and monoclonal antibodies with applications

The main function of the humoral immune response is the destruction of extracellular pathogens (e.g. extracellular bacteria) and the prevention of spread of intracellular pathogens (e.g. intracellular bacteria and viruses) as they move from cell to cell through the extracellular fluids. This is achieved by molecules known as **antibodies** or **immunoglobulins** (Igs).

The first stage in the production of antibodies is activation of the resting naïve B-lymphocytes and their differentiation into antibody-secreting plasma cells. Certain receptors (Fig. 12) are expressed on the surface of the B cells and are essential for their activation. These are:



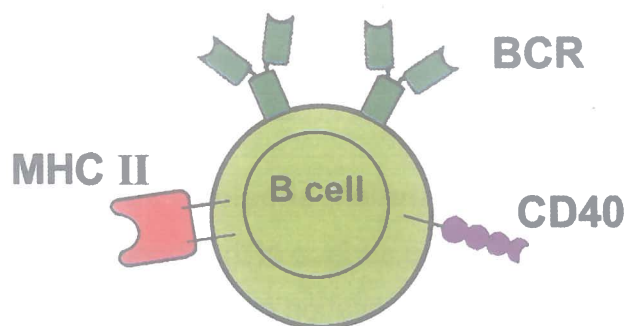


Fig. (12): B cell surface molecules

- a- B cell receptor (BCR):** This is a membrane-bound antibody molecule, also called surface immunoglobulin, that acts as an antigen receptor. All BCRs on a single B cell are of identical specificity and a B cell that binds antigen through this molecule will then secrete antibodies of the same specificity. Immature B cells express only IgM on their surface, whereas mature B cells bear both IgM and IgD.
- b- CD40:** These molecules are essential for the interaction between B and T cells.
- c- MHC II:** These molecules are essential for antigen presentation to T helper cells.

### Sequence of events in activation of naïve B cells: (Fig. 13)

B cell activation is a multi-step process that occurs as follows:

- The mature naïve B cell leaves the blood stream and enters a secondary lymphoid organ (e.g. lymph node). Upon entry, the B cell migrates into a region rich in T cells.
- In the absence of its target antigen, the B cell traverses this region rapidly and eventually re-enters the circulation.
- In the presence of its target antigen, the B cell binds its specific antigen via the surface Igs (BCR). Those B cells that have bound antigen are selectively trapped before leaving the T cell zone, where full activation of the B cells occurs.
- To become fully activated, naïve B cells must receive 2 signals:
  - The first signal is delivered by binding of the antigen to BCR.
  - The second signal (called accessory or co-stimulatory signal) is delivered by activated helper T cells (mainly Th2). To receive this signal, the B cell engulfs the bound antigen, degrades it into peptides and presents these peptides on the cell surface in association with MHC class II molecules. The peptide-MHC class II complex can then be recognized by an antigen-specific helper T cell. This triggers the T cell to produce:
    - a- CD40 ligand (CD40L), which is a T cell surface molecule that binds to the B cell surface molecule CD40
    - b- IL-4, IL-5 and IL-6, which are B cell stimulatory cytokines

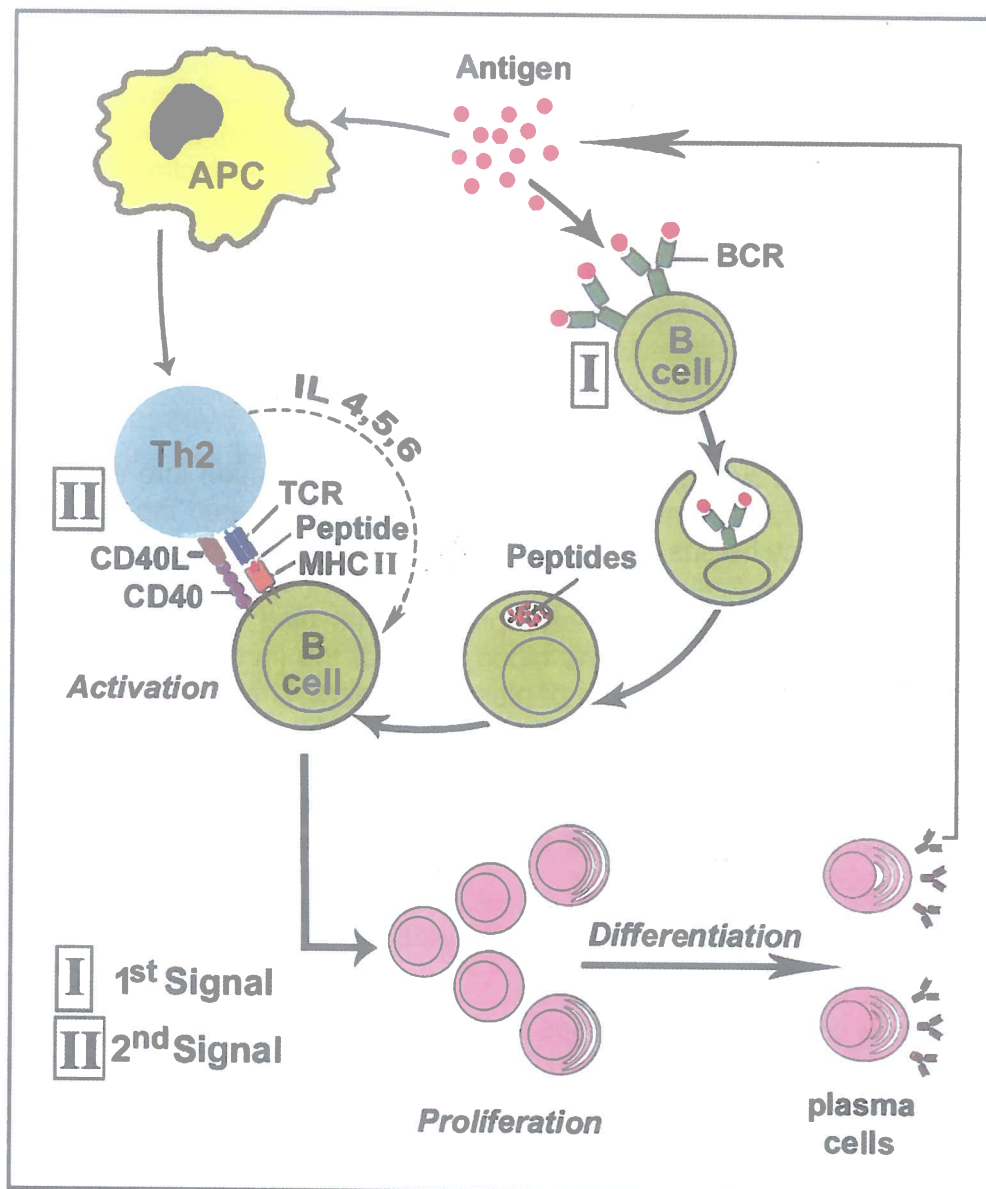


Fig. (13): Activation of naïve B cells

- The B cell is now stimulated to become a lymphoblast (**activation**), and to divide repeatedly (**proliferation or clonal expansion**). Its progeny (daughter cells) become effector cells, which are antibody-secreting plasma cells (**differentiation**). IgM is first secreted, and then the B cell can switch to the production of another isotype of the same immunologic specificity.
- Some B-lymphocytes do not undergo this terminal differentiation. Instead, they become **memory cells** which are long-lived and are the major cells responsible for immunological memory. This sequence of events is known as the **primary immune response**. Most antigens require this form of B cell - T cell collaboration to initiate an immune response and are, therefore, termed **thymus-dependent (TD) antigens**.

- Some antigens, such as bacterial polysaccharides, can activate B cells directly in the absence of T cell help. These antigens are called **thymus-independent (TI) antigens**. In such cases, B cell activation is delivered by the antigen only. TI responses provide an early and specific antibody response against many important bacterial pathogens without the need for T cell activation. In this case:
  - Only IgM is produced and the B cell cannot switch to production of other isotypes.
  - Memory cells are not produced.

## IMMUNOGLOBULINS

After the proliferation of the activated B cells and their differentiation into antibody-secreting plasma cells, surface immunoglobulins (BCR) disappear, and the secretion of large amounts of antibody begins.

Antibodies or immunoglobulins (Igs) are glycoproteins that bind specifically to the antigen that induced their formation. In the blood, most of the immunoglobulins are present within the gamma globulin fraction of plasma proteins. They are also present in the extravascular compartment, e.g. lymph and tissue fluids.

There are 5 classes or isotypes of Igs, namely, IgG, IgA, IgM, IgE and IgD. Within certain classes, there are subclasses that show slight differences in structure and function, e.g. IgG1, IgG2, IgG3 and IgG4.

### Antibody Structure: (Fig. 14)

The basic structure is common to all classes of Igs. An antibody molecule is roughly Y-shaped and consists of 2 identical light (L) and 2 identical heavy (H) polypeptide chains linked together by disulphide (S-S) bonds.

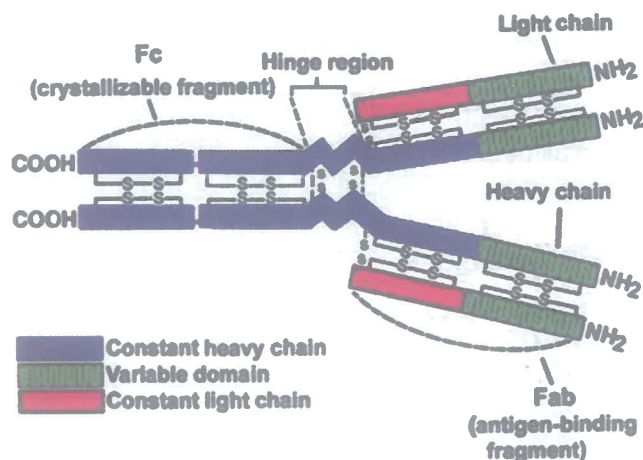


Fig. (14): Basic structure of immunoglobulin molecule



**The light chains**

Each light chain is composed of approximately 200 amino acids and has a molecular weight of about 25 kDa. They are one of two types, kappa ( $\kappa$ ) or lambda ( $\lambda$ ). Both types can be found in all classes of Igs, but only one type is found in one antibody molecule.

**The heavy chains**

Each heavy chain is composed of approximately 400 amino acids, which is twice the number in the light chain, and has a molecular weight of 50-75 kDa, which is also approximately twice that of the light chain. The heavy chains determine the isotype of an Ig. There are 5 main types: gamma ( $\gamma$ ), alpha ( $\alpha$ ), mu ( $\mu$ ), delta ( $\delta$ ) and epsilon ( $\epsilon$ ), corresponding to the 5 isotypes of Igs IgG, IgA, IgM, IgD and IgE respectively. The two heavy chains are joined by a number of S-S bonds in the region known as the hinge region.

**Domains**

- The light and heavy chains are subdivided into regions or domains, each formed of around 110 amino acids.
- These regions are:
  1. Variable regions or domains, which show a wide variation in amino acid composition according to antibody specificity.
  2. Constant regions or domains, which demonstrate a much more uniform (constant) amino acid sequence.
- The light chain consists of one variable (VL) and one constant domain (CL). The heavy chain consists of one variable (VH) and 3 or 4 constant domains (CH<sub>1</sub>, CH<sub>2</sub>, CH<sub>3</sub>, CH<sub>4</sub>).

**The amino (NH<sub>2</sub>)-terminal of the antibody molecule:** It is formed of the variable domains of heavy and light chains (VH, VL). They constitute the antigen-binding sites. Since the antibody has two identical light chains and two identical heavy chains, each antibody will have two identical antigen-binding sites. This allows the antibody molecule to cross-link antigens.

**The carboxyl (COOH)-terminal of the antibody molecule:** It is formed of the constant domains of both heavy chains. It is the same for all members of the same isotype, and determines the functional properties of a particular isotype.

**Hypervariable regions**

The variability in amino acid sequence in the variable domains of light and heavy chains is not spread evenly over their entire length, but is restricted to short segments. These segments show considerable variation and are termed hypervariable regions. The hypervariable regions of heavy and light chains are folded and brought together, creating a single hypervariable surface or **paratope**. The paratope is the antigen-binding site; it is complementary to and interacts with the epitope of the antigen (Fig. 15).

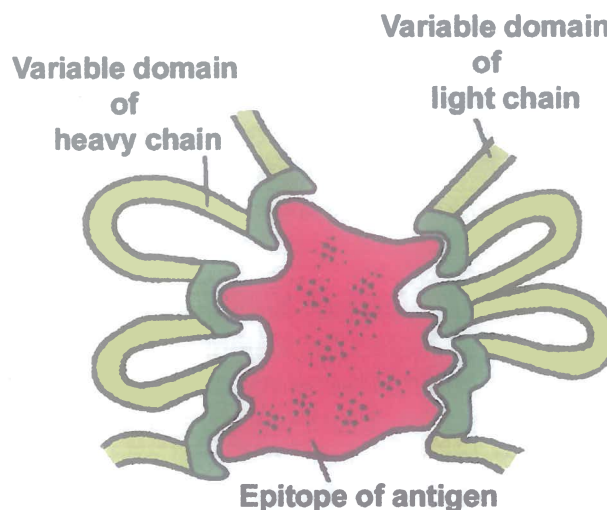


Fig. (15): Hypervariable region

**Proteolytic cleavage:** (Fig. 16)

If an antibody molecule is treated with a proteolytic enzyme, peptide bonds in the hinge region are broken. This produces:

- Two identical **Fab** fragments (fragment antigen binding) which carry the antigen-binding sites, and
- One **Fc** fragment (fragment crystallizable, because it crystallizes easily) which is involved in the biological activities of the antibody molecule such as complement fixation, placental transfer and attachment to various cells with Fc receptors. The Fc fragment differs in antibodies of different isotypes; therefore, different isotypes differ in their biological functions.

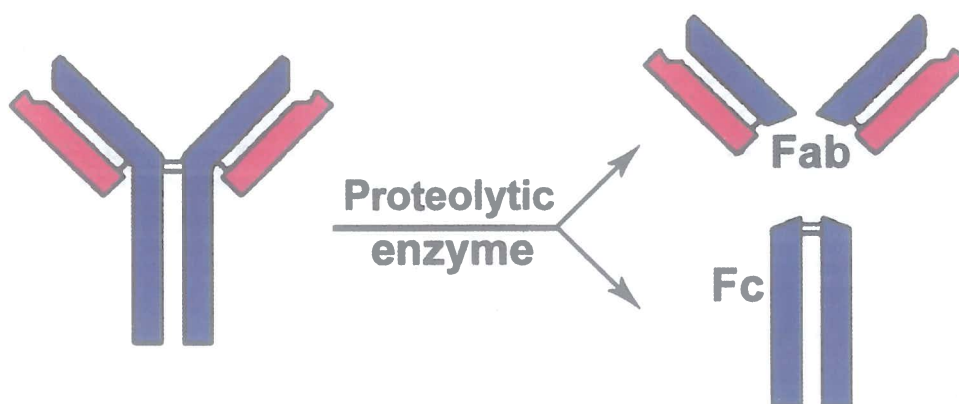


Fig. (16): Proteolytic cleavage of immunoglobulin molecule

## Functions of Antibodies

There are several ways in which antibodies contribute to immunity:

1. **Agglutination:** Binding of antibodies to a particulate antigen (e.g. bacteria) results in clumping of the pathogen which prevents its dissemination and stimulates its removal by other mechanisms (e.g. phagocytosis) (Fig. 17).



Fig. (17): Agglutination by antibodies

2. **Neutralization:** Antibodies can inhibit the infectivity of a pathogen (viruses or bacteria) or the toxicity of a toxin molecule by binding to them, thereby preventing their attachment to their specific receptors on their target cells (Fig. 18).

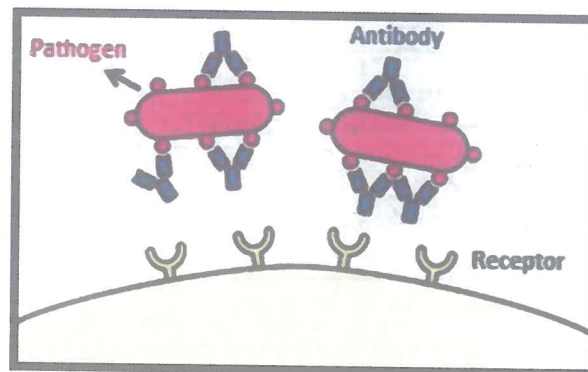


Fig. (18): Neutralization by antibodies

3. **Opsonization:** Phagocytic cells have Fc receptors on their surface that can recognize and bind Fc portion of antibody molecules coating a pathogen. This facilitates the engulfment and subsequent intracellular killing of the pathogen by the phagocytic cells (Fig. 19).

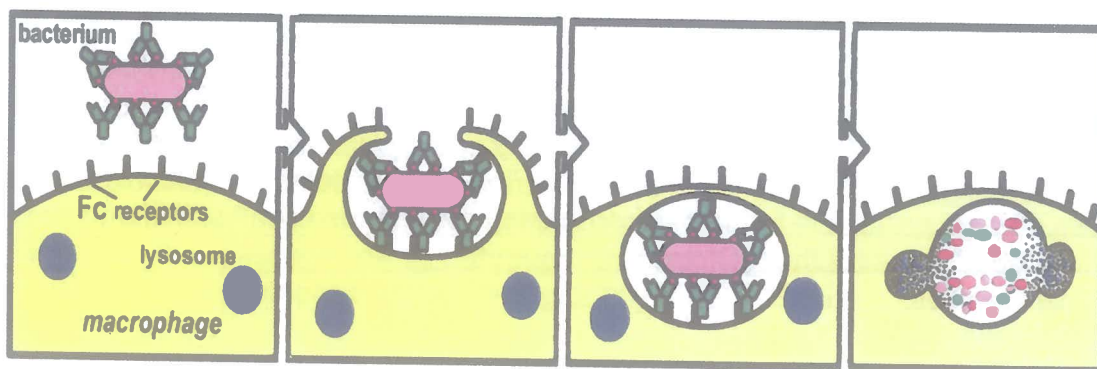


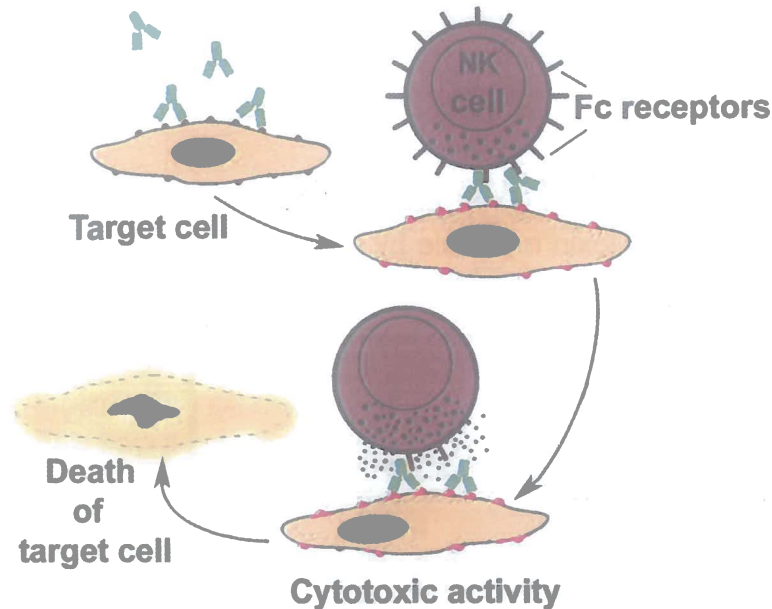
Fig. (19): Antibody-mediated opsonization



**4. Complement activation:** Antibodies bound to the surface of a pathogen may activate proteins of the complement system. This has many consequences (see chapter 7).

**5. Antibody-dependent cell-mediated cytotoxicity (ADCC):** (Fig. 20)

It is the destruction of antibody-coated cells by natural killer (NK) cells. NK cells possess receptors for the Fc portion of antibodies. An antibody bound to an antigen on a target cell can also bind to the NK cell through its Fc portion, facilitating adhesion of the NK cell to the target cell and triggering its cytotoxic activity. Other cells possessing Fc receptors, e.g. macrophages, may also exert ADCC.



**Fig. (20): Antibody-dependent cell-mediated cytotoxicity (ADCC)**

**N.B.:** The type of the antibody (isotype) produced determines which effector mechanism occurs in a particular immune response.

## Immunoglobulin Classes (Isotypes)

### I. Immunoglobulin G (IgG)

- IgG is composed of a single basic unit (monomer).
- There are 4 subclasses (IgG<sub>1</sub>-IgG<sub>4</sub>) based on H chain differences.
- It is the principal isotype in blood (75% of circulating Igs) and extracellular fluids.
- It is the major antibody of the secondary immune response.
- Placental transfer: IgG interacts with Fc receptors in the placenta and is, therefore, the only Ig that can pass the placental barrier to the foetal circulation. This provides passive protection to the newborn during the first few months of life.
- Anti-Rh antibodies are of the IgG class.
- Biological activities:
  - Neutralization
  - Opsonization
  - Complement activation
  - ADCC

## II. Immunoglobulin M (IgM)

- IgM is composed of 5 basic units (pentamer) held together by disulphide bonds and a short peptide chain (joining or J chain) (Fig. 21).
  - Because of its large size, IgM is mainly confined to the blood (8-10% of circulating Igs).
  - It is the major antibody of the primary immune response.
  - It is the only antibody made to thymus-independent antigens (e.g. ABO blood group antigens of human RBCs).
  - IgM cannot cross the placenta; therefore, its presence in the newborn blood indicates intrauterine infection.
  - Biological activities:
    - Agglutination
    - Complement activation
- IgM is the most efficient agglutinating and complement-fixing antibody.
- IgM is found on the surface of B cells forming BCR.

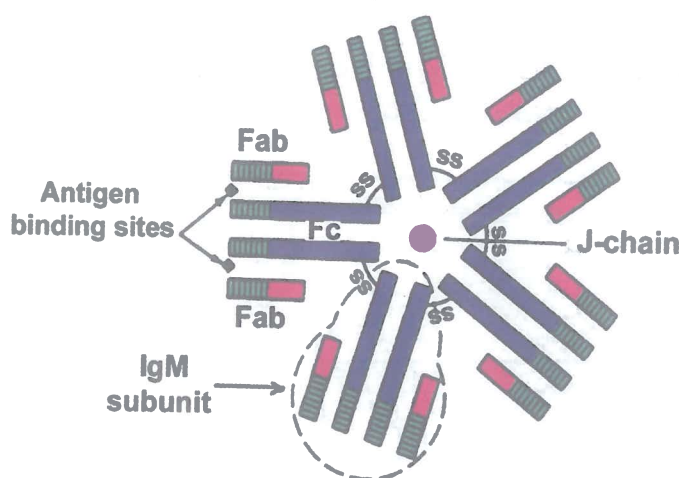
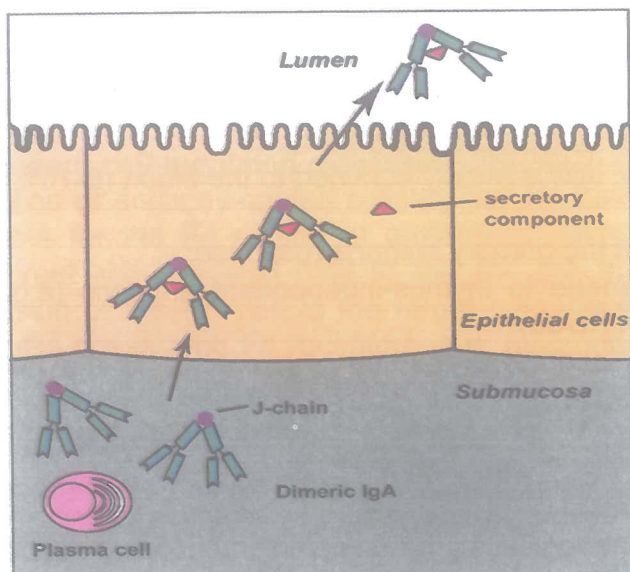


Fig. (21): Immunoglobulin M (IgM) molecule

## III. Immunoglobulin A (IgA)

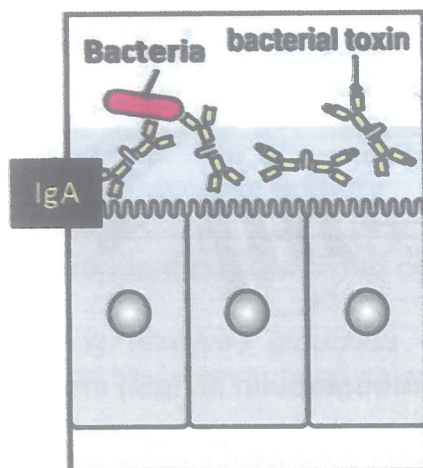
This class of immunoglobulins exists in two forms:

1. Monomeric form (serum IgA), which is found in serum. It represents about 15-20% of total serum immunoglobulins and its function is uncertain.
2. Dimeric form (secretory IgA), which is produced by the submucosal plasma cells and is found in the mucosal secretions (saliva, tears, colostrum, respiratory, GIT and genitourinary secretions). The dimeric form of secretory IgA is composed of two basic units, a J chain that joins the two units together, and a secretory component. The secretory component is synthesized by local mucosal cells. It facilitates the passage of IgA through the epithelial cells and protects the molecules from proteolytic digestion (Fig. 22).



**Fig. (22): Secretory IgA**

Secretory IgA provides local immunity at the mucosal surfaces. Its main function is neutralization as it prevents attachment of organisms to mucosal surfaces (Fig. 23).



**Fig. (23): Neutralization by IgA at mucosal surface**

#### IV. Immunoglobulin D (IgD)

- IgD exists as a monomer.
- It accounts for less than 1% of circulating antibodies.
- It is found on the surface of mature B cells where it acts as an antigen receptor (BCR).

#### V. Immunoglobulin E (IgE)

- IgE exists as a monomer.
- It is present in trace amounts in serum.
- It is also present attached to Fc receptors on mast cells and basophils and plays an important role in type I hypersensitivity reactions.



- IgE binding to Fc receptors on eosinophils is important in immunity to parasitic worms, as it triggers eosinophils to release toxic substances on the surface of the parasite.

### Immunoglobulin Class Switching (Isotype Switching): (Fig. 24)

During the immune response, plasma cells switch from producing IgM to produce IgG or other immunoglobulin classes. Class switching is mediated by a change in the constant domains of the H chain (CH). There is no alteration in the L chain or the variable domain of the heavy chain (VH), so that the immunoglobulin produced later (IgG, IgA or IgE) has the same specificity as the original IgM but differs in the biological characteristics.

Class switching is dependent on cytokines released from T cells:

- Under the effect of IL-4 alone the expanded B-cell clones can differentiate and mature into IgE-secreting cells.
- TGF- $\beta$  (transforming growth factor- $\beta$ ) encourages cells to switch their Ig class to IgA. IL-5 then augments IgA production by these cells.
- Plasma cells produce large amounts of IgG under the combined influence of IL-4, IL-5 and IL-6.

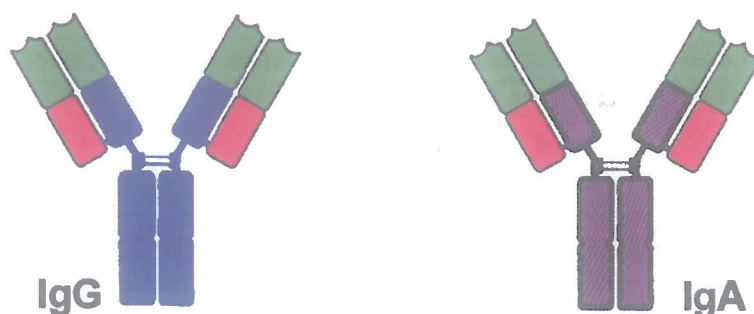


Fig. (24): Immunoglobulin class switching

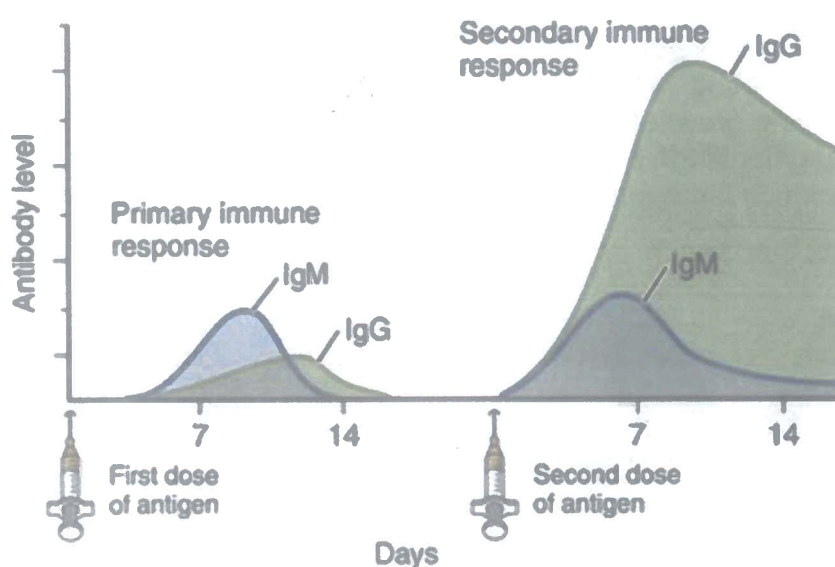
### Primary and Secondary Antibody Response: (Fig. 25) (Table 10)

Primary antibody response occurs when an antigen is introduced for the first time. There is a lag (induction) period, during which antibody cannot be detected (7-10 days). This is the time needed for B cells to undergo activation, proliferation and differentiation into antibody-secreting plasma cells. The isotype produced is mainly IgM. With time, various mechanisms turn off the response e.g. removal of the antigen. Then antibody production is stopped and there is rapid decline in its concentration to undetectable levels.

Secondary antibody response occurs on subsequent exposure to the same antigen. Memory cells (generated in the first exposure) are readily activated, and antibodies can be detected after a short lag period. IgG is the main isotype produced. The antibody level is 10 times greater than during the primary response and is maintained at high levels, falling slowly over a period of months. The response can be boosted to higher levels by further exposures. For this reason, most vaccines are given in more than one dose (booster doses).

**Table (10):** Comparison between primary and secondary antibody response:

	Primary response	Secondary response
Induction (lag) period	Long (7-10 days)	Short (few hours to few days)
Antibody level	Low	High (10 times greater)
Duration	Short (antibodies decline rapidly)	Long (months)
Ig class	Predominantly IgM	Predominantly IgG
Memory cells	Develop at the end	Present from the start

**Fig. (25):** Primary and secondary antibody response

### Heterophil Antibodies

Because of the similarity that may be found between different antigens (sharing of epitopes), antibodies produced in response to an antigen may cross-react with another one. Such cross-reacting antibodies are called "heterophil antibodies".

### Monoclonal Antibodies

These are highly specific antibodies against a single epitope produced by a single clone of B cells. They can be artificially produced to be used in diagnosis and therapy.

#### a- Diagnostic applications

Monoclonal antibodies are widely used in different kinds of serological reactions for antigen detection.

Example: determination of lymphocyte markers (e.g. CD markers).

#### b- Therapeutic applications

Monoclonal antibodies are used in treatment of some conditions.

Example: treatment of drug toxicity, such as digitalis toxicity.

**MCQs:**

- 1- **For full activation, naïve B cells must receive the following signal(s):**
  - a- Signal derived from antigen binding to BCR
  - b- Signal derived from activated cytotoxic T cells
  - c- Signal derived from activated helper T cells
  - d- a & b
  - e- a & c
- 2- **Thymus independent (TI) antigens:**
  - a- Can stimulate B cells through T cell help
  - b- Do not produce memory cells
  - c- Can stimulate immunoglobulin class switching
  - d- Can only induce the production of IgG
  - e- Are usually protein in nature
- 3- **The portion of the antibody molecule that binds antigenic epitopes is:**
  - a- Termed the determinant
  - b- Composed of variable and constant regions of Ig heavy and Ig light chains
  - c- Composed of the variable regions of Ig heavy and Ig light chains
  - d- The Fc fragment
  - e- Two Ig light chains
- 4- **IgM:**
  - a- Can cross the placenta
  - b- Has many J chains
  - c- Is the predominant antibody in the secondary immune response
  - d- Indicates immunity when present in the newborn
  - e- Is the only immunoglobulin to thymus independent antigens
- 5- **IgE:**
  - a- Is usually present as a dimer
  - b- Is present in large amounts in serum
  - c- Is present as B cell receptor on B cells
  - d- Can attach to receptors on mast cells
  - e- Can cross the placenta
- 6- **Immunoglobulin class switching is mediated by a change in:**
  - a- Constant domains of heavy chains
  - b- Constant domains of light chains
  - c- Variable domain of heavy chain
  - d- Variable domain of light chain
  - e- Constant domains of both heavy and light chains
- 7- **Regarding the primary immune response:**
  - a- There is a very short induction (lag) period.
  - b- Immunoglobulins produced are mainly of IgM class.
  - c- Memory cells are the main effector cells.
  - d- The induced antibody level is very high.
  - e- It is the first response of innate immunity.



## COMPLEMENT

### ILOs:

By the end of this chapter the student should be able to:

- Describe the mechanisms by which the complement system is activated and regulated.
- Compare and contrast between the various complement activation pathways
- Describe role of complement in immune mechanisms
- Explain how and why complement system has to be regulated

The complement system is a group of heat-labile proteins normally found in blood and tissue fluids (except urine and CSF).

- These proteins are termed complement factors because they are required to 'complement' the bactericidal effects of antibodies.
- They are mainly produced by the liver, mostly in an inactive form.
- The basic complement proteins are termed C1 to C9, in addition to factor B, D and properdin and some complement regulatory proteins. Activation of complement occurs through interaction of complement factors in a sequential manner, one step after the other. The product of one reaction forms the enzyme for the next, and so on. This mode of activation is called the "complement activation cascade".
- When they become activated, some complement factors are split into a small fragment (a) which is considered a by-product, and a large fragment (b) which continues the activation process.

### Complement Activation

- There are 3 pathways for complement activation: the classical pathway, which is triggered by antibodies, and the lectin and the alternative pathways which are initiated in the absence of antibody.
- The *early steps* in all pathways involve a series of cleavage reactions. These end with the production of C3b that becomes attached to the microbial surface and initiates the *late steps* of the complement cascade.
- Thus, the 3 pathways differ in how they are initiated (early steps), but they share the late steps and also perform the same effector functions.

### A) Early Steps of Complement activation

#### 1. Via the classical pathway (Fig. 26):

- This pathway starts by binding of the first complement component (C1) to the Fc portion of either IgG or IgM attached to the antigen (e.g. a bacterial cell).
- This causes activation of C1, followed by activation of C4, C2 and C3 (in that order), which involves cleavage of these components into a and b fragments.
- The formed C3b molecules bind directly to the microbial surface, which becomes saturated with C3b.

**2. Via the lectin pathway:**

- This pathway is activated when a plasma protein, mannose-binding lectin (MBL), binds to mannose residues on microbial surface.
- MBL is structurally and functionally similar to C1. Thus, activation of C4, C2 and C3 follows as in the classical pathway, ending in the deposition of large numbers of C3b on the pathogen surface.

**3. Via the alternative pathway:**

- C3 is abundant in plasma and C3b is produced continuously by its spontaneous cleavage.
- If C3b becomes deposited on a microbial surface it can bind to microbial surface components, such as bacterial endotoxins, yeast cell wall and viral envelopes.
- This triggers the activation of the early steps of the alternative pathway. Involvement of factor B, factor D and properdin allows cleavage of more C3 resulting in the deposition of large numbers of C3b on the pathogen surface.

**B) Late Steps of Complement activation**

- The late steps of complement activation are the same for the three pathways.
- They start by cleavage of C5 into C5a and C5b.
- C5b binds to the terminal complement components C6, C7, C8 and C9 sequentially to form a complex called membrane attack complex (MAC).
- This complex (C5b6789) forms a hollow cylinder that becomes inserted into the target cell membrane, allowing free passage of water and solutes across the membrane. This leads to cell death (osmotic lysis).

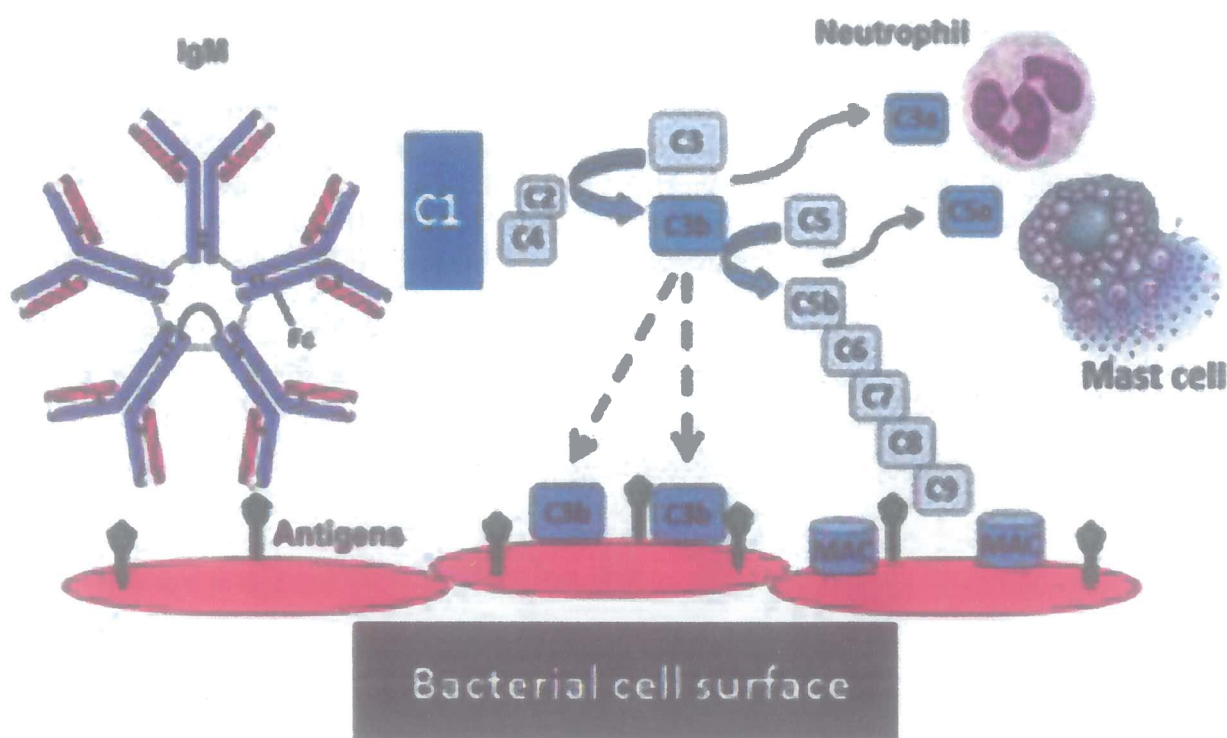


Fig. (26): Classical pathway of complement activation

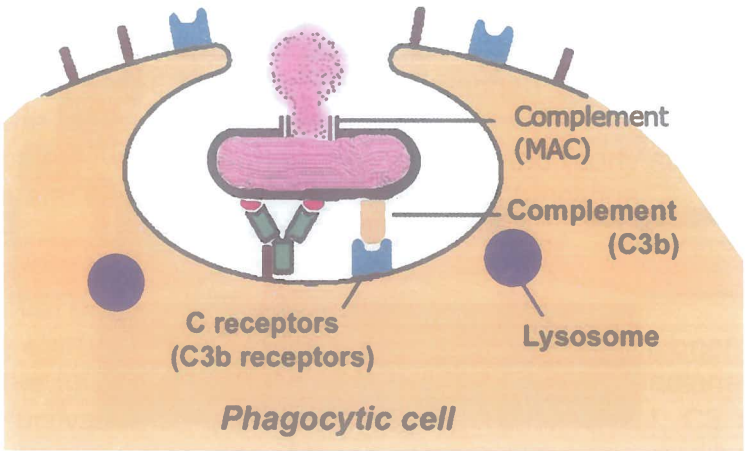
- ❖ Antibodies have no role in the activation of complement via the alternative or lectin pathways. Therefore, these pathways are considered to be mechanisms of innate immunity. On the other hand, the classical pathway requires the presence of antibodies and is, therefore, a part of the acquired immune response (Table 11).
- ❖ If C3b becomes deposited on a host cell surface, it is prevented from binding stably by several regulatory proteins that are present on host cells but absent from microbes. Thus triggering of activation of the complement system on host cells and their subsequent lysis is prevented.

**Table (11):** Comparison between the 3 pathways of complement activation:

	Classical pathway	Lectin pathway	Alternative pathway
Type of immunity	Acquired (specific)	Innate (non-specific)	Innate (non-specific)
Initiation	Antigen-antibody complex	Lectin binding to pathogen surface	Deposition of C3b on microbial surface
Role of antibodies	Needed for initiation (activation of C1)	Have no role	Have no role
Role of factors B, D and P	Have no role	Have no role	Have a role
Role of MBL	Has no role	Has a role	Has no role
The involved components	C1,4,2,3,5,6,7,8,9	C4,2,3,5,6,7,8,9	C3,5,6,7,8,9

Functions of Complement

- 1. Direct cytolysis:** Insertion of the MAC into the cell surface leads to killing of many cells, e.g. bacterial and tumour cells, through osmotic lysis (Fig. 27).
- 2. Opsonization:** During complement activation, C3b becomes deposited on the surface of the pathogen (antigen). Phagocytic cells recognize C3b bound to the pathogen via their C3b receptors. This facilitates the attachment and subsequent uptake and killing of the C3b-coated pathogen by the phagocytic cell (Fig. 27). This is another example of opsonization, besides opsonization by antibodies previously mentioned.



**Fig. (27):** Complement-mediated opsonization and bacterial cell lysis



**3. Immune complex clearance:** RBCs possess C3b receptors, which recognize C3b bound to soluble immune complexes. RBCs bind the immune complexes via these receptors and transport them to organs rich in fixed phagocytes (e.g. liver and spleen). Using their own C3b and Fc receptors, these phagocytes remove the immune complexes from the red cells. This helps clearance of soluble immune complexes from the circulation and prevents the development of immune complex diseases (Fig. 28).

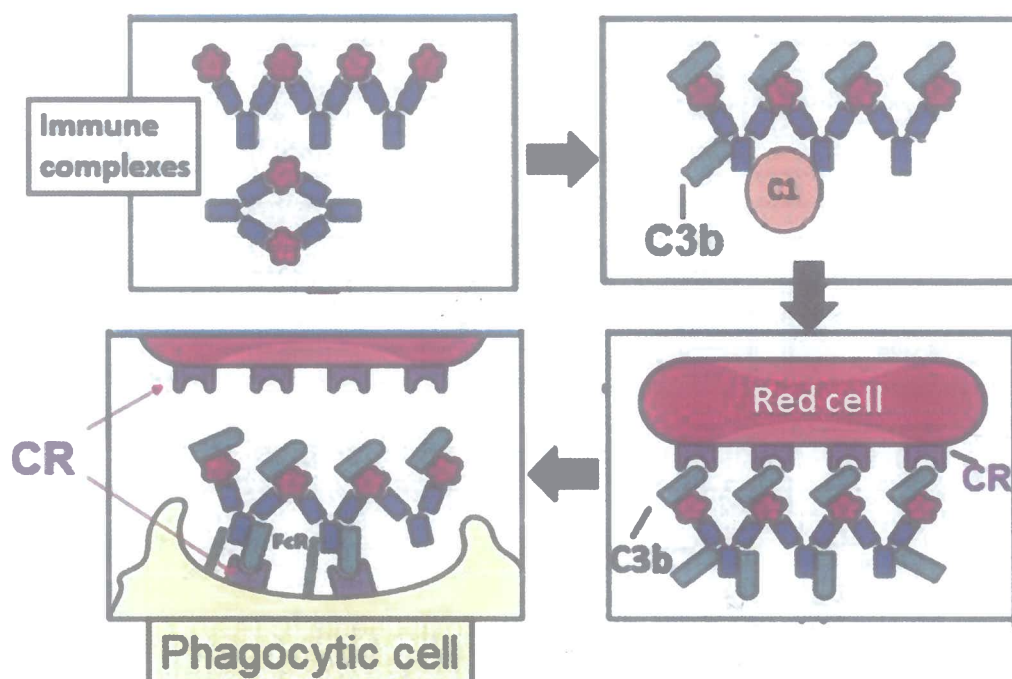


Fig. (28): Removal of immune complexes

**4. Inflammatory response:** During complement activation, the by-products C3a and C5a are produced. These molecules, known as anaphylatoxins, have the following important biological activities:

- Degranulation of mast cells and basophils to release mediators of inflammation, e.g. histamine
- Recruitment of phagocytic cells to the site of inflammation (chemotaxis) and stimulation of their phagocytic power and intracellular killing

### Regulation of the Complement System

- Complement tends to undergo spontaneous activation, especially by the alternative pathway. The activated complement components can destroy any cell to which they bind.
- Host cells are protected from such damage by a series of complement-regulatory proteins. Some of these proteins are associated with the host cell surface, whereas others are plasma proteins.
- They may cause degradation or inactivation of activated complement components. One example is C1 inhibitor, which binds to and inactivates C1 preventing further cleavage of C4 and C2.
- Deficiency of regulatory proteins results in excessive complement activation that causes inflammation and widespread tissue damage.

A schematic representation of the complement pathways and functions is shown in Fig. (29).

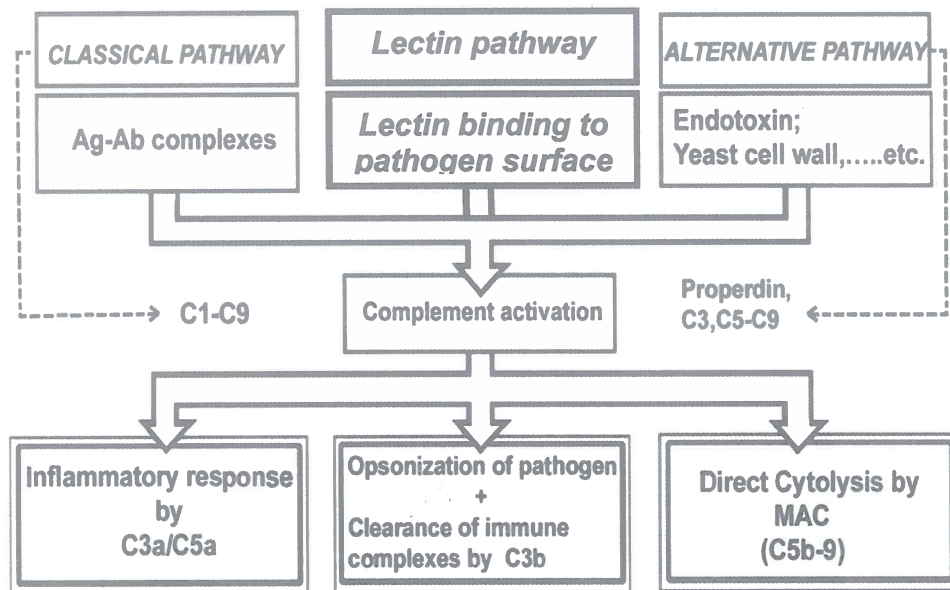


Fig. (29): Complement pathways and functions

#### MCQs:

- 1- In the complement system:
  - a- The alternative pathway involves the nine components (C1-C9).
  - b- The alternative pathway is activated by mannose-binding lectin.
  - c- The classical pathway is best activated by bacterial endotoxin.
  - d- The membrane attack complex is made of C5b-C9.
  - e- The early steps in complement activation are similar in the 3 pathways.
- 2- All of the following are functions of activated complement EXCEPT:
  - a- Bacterial lysis
  - b- Opsonization
  - c- Interference with viral replication
  - d- Chemotaxis
  - e- Immune complex clearance
- 3- The complement component which attaches to the Fc portion of IgM is:
  - a- C3a
  - b- C3b
  - c- C5a
  - d- C5b
  - e- C1
- 4- Anaphylatoxins:
  - a- Have chemotactic effect on phagocytic cells
  - b- Attach to C3b receptors on phagocytic cells
  - c- Play an important role in immune complex clearance
  - d- Can induce cytolysis
  - e- Include properdin

## CLASSIFICATION OF ACQUIRED (SPECIFIC) IMMUNITY

### **ILOs:**

**By the end of this chapter the student should be able to:**

- Classify acquired immunity against various infectious agents
- Recognize the ways of acquiring specific immune response
- Compare between active and passive immunity
- Define vaccination
- Classify the types of vaccines against infectious organisms
- Differentiate between different types of vaccines and highlight the advantages and disadvantages of each type
- Summarize the new techniques of vaccine production

Specific immunity against various infectious agents may be acquired either passively or actively (Fig.30) (Table 12).

### **I. Active Acquired Immunity**

Antigens of the microorganism must come in contact with cells of the immune system, resulting in specific stimulation of B and/or T cells. This form of immunity takes some time to develop (time needed to induce activation, proliferation and differentiation of lymphocytes). However, immunological memory lasts after the antigen is eliminated, allowing a quicker and more efficient response following subsequent exposure to the same antigen.

There are two ways for acquiring active immunity:

#### **A- Natural active immunity**

This occurs following natural infections, whether clinical or sub-clinical. Some infections, such as measles, induce long-lasting immunity. Others, such as influenza, confer immunity lasting for a relatively shorter time.

#### **B- Artificial active immunity**

This occurs by deliberate administration of microorganisms or their products (in an unharmed state) into a host, a process called **vaccination** or **active immunization**. The aim is to produce an appropriate immune response which is capable of protecting the host when he is later exposed to a virulent form of the same pathogen. Various vaccines are used routinely to protect people from infectious diseases.



## II. Passive Acquired Immunity

This involves transfer of ready-made antibodies and/or lymphocytes to an individual. The immune system has no active role in initiating an immune response. Immunity acquired here is immediate, but is temporary, remaining only as long as the transferred material remains active in the recipient's body. There are two ways for acquiring passive immunity:

### A- Natural passive immunity

This is the transfer of maternal antibodies (IgG) to the foetus across the placenta, or passage of secretory IgA to the newborn in the colostrum. This protects the newborn against many diseases during the first six months of life.

### B- Artificial passive immunity

- **Humoral immunity** can be artificially transferred to an individual by transfer of antibodies. This is called **passive immunization**. Examples include:
  - Administration of **antitoxic serum** for treatment against infections caused by bacteria that produce exotoxins, as in diphtheria and tetanus. (Antitoxic serum is obtained by repeated injection of human volunteers or animals with a toxoid, provoking production of antibodies against it, so that their serum is rich in that specific antitoxin and can be administered to another individual).
  - Administration of **gamma globulin** to an immunodeficient person. (Gamma globulin is pooled serum obtained from normal adults and contains a mixture of many protective antibodies).
  - Administration of **convalescent serum** to exposed people to protect from diseases such as hepatitis A infection. (Convalescent serum is serum obtained from a person convalescing from a certain infectious disease and is, therefore, rich in specific antibodies).
- **Cell-mediated immunity** can be transferred by transfer of lymphocytes. However, such cell transfer must be between genetically identical individuals to avoid rejection reactions. It is, therefore, not suitable for clinical use, but is used experimentally in genetically identical animals.

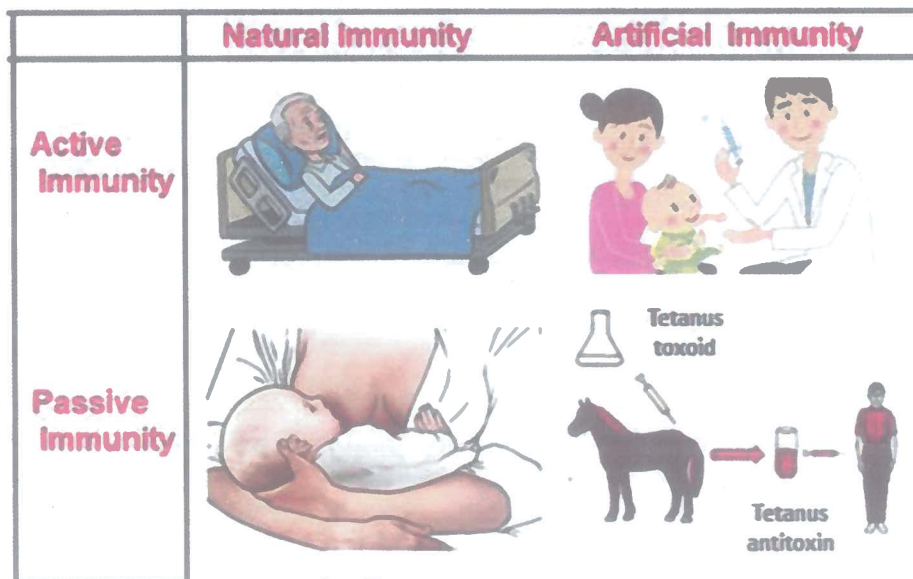


Fig. (30): Types of acquired immunity

**Table (12):** Comparison between active and passive immunity:

	Active immunity	Passive immunity
<b>Role of immune system</b>	Important role	No role
<b>Mechanism</b>	Stimulation of B/T cells	Transfer of ready-made antibodies/lymphocytes
<b>Onset of protection</b>	Delayed	Immediate
<b>Duration of protection</b>	Longer	Shorter
<b>Development of memory</b>	Yes	No
<b>Examples</b>	-Natural infections -Active immunization with vaccines	-Maternally-acquired antibodies -Passive immunization with antitoxic serum

## VACCINATION

Pathogens used in vaccines may be introduced whole, in a killed or attenuated form. Alternatively, only parts or products of the organism are used. The form of the vaccine determines the kind of immune response produced:

- Non-living vaccines cannot enter the MHC class I pathway and, therefore, cannot induce cytotoxic T cells.
- Live vaccines, on the other hand, have access to both MHC class I and II pathways and can, therefore, induce both cytotoxic and helper T-cells. In addition, replication of living organisms increases the stimulation of the immune system and mimics the natural disease more closely.

Live vaccines are, therefore, more effective in inducing a better overall immune response. However, they cannot be used in pregnant women and immunocompromised hosts. They also require refrigeration, which makes them more expensive and difficult to use in underdeveloped countries.

Vaccines, in general, can be classified as follows:

## A- Bacterial Vaccines

### 1. Intact Bacteria:

- a- **Killed:** using organisms killed by heat or chemicals, e.g. pertussis killed whole-cell vaccine.
- b- **Live attenuated:** prepared by frequent subculture on artificial suboptimal media, e.g. Bacille Calmette-Guerin (BCG) vaccine against tuberculosis.

### 2. Bacterial Materials:

- a- **Structural Components:** such as the capsule of bacteria, which induce the production of anti-capsular antibodies that neutralize the antiphagocytic effect of the capsule, e.g. pneumococcal polysaccharide vaccine.
- b- **Toxoids:** Non-toxic, immunogenic preparations produced from exotoxins by the use of formalin. They induce antitoxic antibodies. Well known toxoids are diphtheria and tetanus toxoids.

## B- Viral Vaccines

### 1. Intact virus

- a- **Killed (inactivated):** Inactivation is done by formalin or ultraviolet light. Examples are Salk polio vaccine and rabies vaccine.
- b- **Live attenuated:** Attenuation is done by repeated subculture in tissue culture or serial passage in various animal hosts. Examples are Sabin polio vaccine, mumps, measles and rubella vaccine (MMR).

### 2. Viral Components

- a- **Isolated viral antigens:** Hepatitis B vaccine is composed of hepatitis B surface antigen (HbsAg).
- b- **Disrupted or split vaccine preparations** such as the influenza vaccine containing the haemagglutinin and neuraminidase.

Progress in molecular biology, as well as a better understanding of immune response to pathogens, has led to **new techniques of vaccine production**.

Examples include:

1. **New methods of attenuation of virus** by deletion of genes responsible for virulence which prevents their reversion to virulence.
2. **DNA vaccines:** This method involves the injection of naked DNA plasmids encoding immunogenic proteins of interest. The protein is produced in the body for a long time and is a constant source of stimulation to both arms of the immune system without a need for booster doses.



**MCQs:**

- 1- In active immunity all the following are true **EXCEPT**:
  - a- The onset of protection is delayed.
  - b- There is development of memory.
  - c- The duration of protection is short.
  - d- There is stimulation of B/T cell.
  - e- It is specific.
- 2- The main advantage of passive immunization over active immunization is that:
  - a- It contains primarily IgM.
  - b- It can be administered orally.
  - c- It provides antibody more rapidly.
  - d- Antibody persists for a longer time.
  - e- Memory persists longer.
- 3- The following represents artificial active acquired immunity:
  - a- Antibodies produced after subclinical infection
  - b- Antibodies produced after vaccination
  - c- Antibodies passing from mother to child
  - d- Antibodies produced after clinically manifest infection
  - e- Administration of antitoxin
- 4- The following represents passive acquired immunity:
  - a- Antibodies produced after subclinical infection
  - b- Antibodies produced after vaccination
  - c- Antibodies produced after clinically manifest infection
  - d- Antibodies produced in response to toxoid
  - e- Antibodies passing from mother to foetus
- 5- Treatment with gamma globulin is considered:
  - a- Artificial active immunity
  - b- Artificial passive immunity
  - c- Natural active immunity
  - d- Natural passive immunity
  - e- Innate immunity

## IMMUNITY TO MICROBES

### ILOs:

By the end of this chapter the student should be able to:

- State the general features of immunity against microbes
- Recognize the main mechanisms of immune response to extracellular bacteria
- Recognize the main mechanisms of immune response to intracellular bacteria
- Recognize the main mechanisms of immune response to viruses
- Recognize the main mechanisms of immune response to fungi
- Identify the main points of major mechanisms by which bacteria and viruses evade the immune system

The principal physiologic function of the immune system is to protect the host against pathogenic microbes.

### General features of immunity against microbes

1. Defence against microbes is mediated by both innate and specific immunity. The innate immune response to microbes plays an important role in determining the nature of the specific immune response. Production of IL-12 by macrophages, for example, leads to development of Th1 cells and consequently a good cell-mediated immune response (Fig. 31).

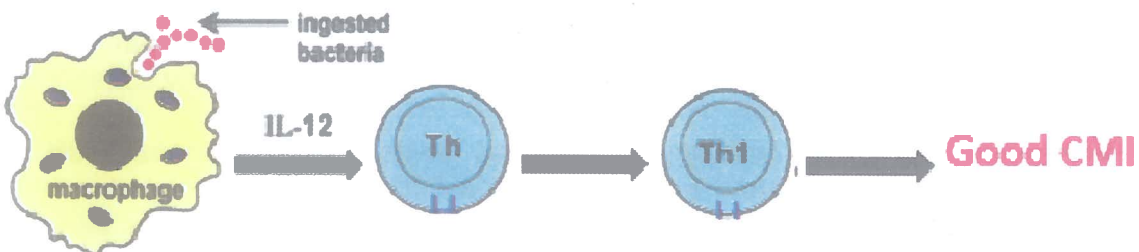


Fig. (31): Innate immune response determines nature of specific immune response

- Specific immunity enhances the protective mechanisms of innate immunity. Examples include the activation of macrophages by  $\text{IFN-}\gamma$  and activation of NK cells by IL-2, both of which are produced by T helper cells (Fig. 32).

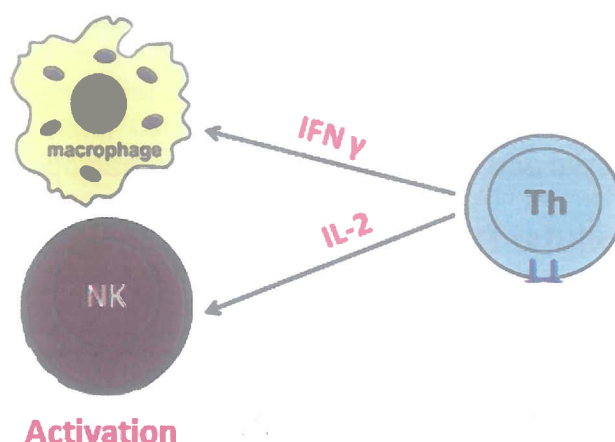


Fig. (32): Enhancement of innate immunity by acquired immunity

- The immune system is capable of responding in different ways to different microbes, in order to combat these agents most effectively.
- The survival of pathogenic microbes in a host depends on the ability of these microbes to evade or resist the body's immune mechanisms.
- During infections, tissue injury and disease may sometimes be caused by the host response to the microbe and its products rather than by the microbe itself.

## I. Immunity to Extracellular Bacteria

Extracellular bacteria are capable of replicating outside host cells. Examples are the pyogenic (pus-forming) cocci as well as some enteric organisms.

### A- Innate Immunity

- Phagocytosis by neutrophils, monocytes and tissue macrophages: Extracellular microbes are rapidly killed by the microbicidal mechanisms of phagocytes.
- Activation of complement by the alternative pathway: Complement can be activated by the peptidoglycan and lipopolysaccharide in the cell wall of bacteria. Complement activation leads to production of opsonins, recruitment and activation of phagocytes and lysis of bacteria.

### B- Specific Immunity

#### • Humoral immune response

This is the **main** protective specific immune response against extracellular bacteria. Antibodies eliminate extracellular bacteria through:

- Opsonization
- Agglutination of bacteria, preventing spreading and facilitating phagocytosis
- Neutralization of bacterial toxins, preventing their binding to target cells, as well as inhibition of adhesion of bacteria to host cells
- Activation of complement by the classical pathway, with all its consequences



- **T cell response**

Extracellular bacteria and their products are internalized by APCs and peptides from them are presented to T cells in association with MHC II molecules. Thus, the main T cell response is that of T helper cells. Their effector functions against extracellular bacteria are mediated by the cytokines they secrete and include:

- Stimulation of antibody production
- Induction of local inflammation
- Enhancement of phagocytic and microbicidal activities of macrophages

## II. Immunity to Intracellular Bacteria

A characteristic of intracellular bacteria is their ability to survive, and even replicate, within phagocytes. This is the reason why these organisms tend to cause chronic infections that last for years and may recur after apparent cure. An example is *Mycobacterium* which causes tuberculosis and leprosy.

### A- Innate Immunity

- Phagocytic cells are usually ineffective in controlling infections caused by intracellular organisms, unless activated by IFN- $\gamma$ .
- NK cells produce IFN- $\gamma$  which activates macrophages. Thus they provide early defence against these microbes before specific immunity develops.

### B- Specific Immunity

- **Humoral immune response**

Since intracellular bacteria are capable of finding a hiding place where they are inaccessible to circulating antibodies, humoral immunity does not play a role in their elimination.

- **T cell response**

*Cell-mediated immunity (in the form of macrophage activation by Th1 cells) is the main protective immune response against intracellular bacteria.* Such bacteria induce macrophages to produce IL-12 which, in turn, promotes the development of Th1 cells. These secrete IFN- $\gamma$  which activates the macrophages to kill the bacteria that they harbour (see Fig. 11, Chapter 5).

Although the development of Th1 cells is very important for dealing with intracellular bacteria, sometimes this does not happen, and this has a great effect on the outcome of an infection with intracellular bacteria. A striking example is seen in the disease leprosy, which is caused by *Mycobacterium leprae*. Macrophage activation by Th1 cells is required for combating this disease. Two forms of the disease are present:

- In **tuberculoid leprosy**, Th1 cells are dominantly induced. The result is that there is intracellular digestion of the mycobacteria and few living bacteria are found in the tissues. Although the skin and peripheral nerves are damaged by the inflammatory responses associated with macrophage activation, the disease is under control and the patient survives.
- In **lepromatous leprosy**, Th2 cells are dominantly induced. The main response is humoral, but this is useless, since the antibodies cannot reach the intracellular

bacteria. The organism grows abundantly in the macrophages causing much destruction and eventually death.

### III. Immunity to Viruses

Viruses are obligate intracellular parasites. They reside in the cytosol of the cell and, thus, peptides from them are presented on the cell surface in conjunction with MHC I molecules. Most viruses, after replication inside the cells, are liberated to infect other cells. On the other hand, certain viruses remain inside the cells they infect and replicate without causing cell lysis.

#### A- Innate Immunity

- NK cells:

Cytotoxic killing of virally infected cells by NK cells is one of the main mechanisms of immunity against viruses early in the course of infection (Fig. 33).

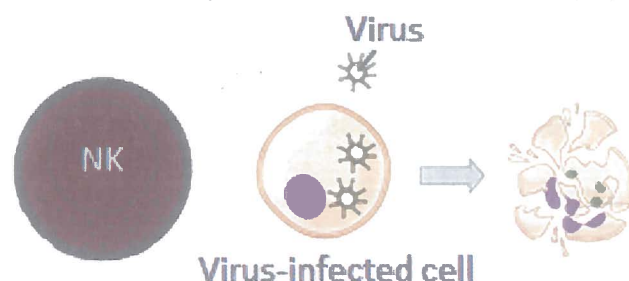


Fig. (33): Cytotoxic killing of a virus-infected cell by NK cell

- Type I interferon:

Virally-infected cells produce type I interferon (IFN- $\alpha$  and IFN- $\beta$ ). These function in many ways to combat viral infections:

- They inhibit viral replication and induce an antiviral state.
- They activate NK cells.
- They increase the expression of MHC I molecules, allowing better presentation of viral peptides to Tc cells.

#### B- Specific Immunity

- Humoral immune response

Specific antibodies are important in defence against viruses before they enter their target cells, and against viruses released from infected cells. This is because in these two situations, virus particles are accessible to antibodies (Fig. 34).

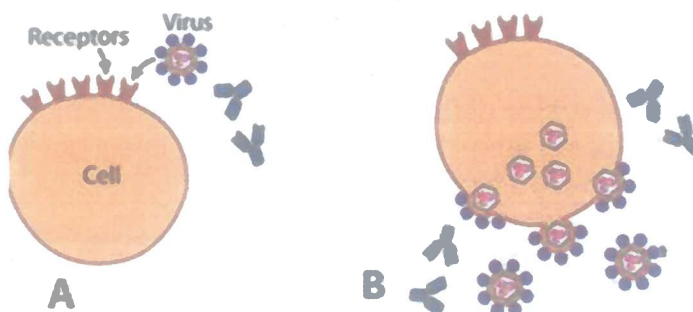


Fig. (34): Antibodies attack viruses (A) before entering cell (B) after release from cell

Antibodies function against viruses in many ways:

1. Neutralizing antibodies bind to viruses and prevent viral attachment and entrance into host cells.
2. Opsonizing antibodies enhance phagocytosis.
3. Activation of complement by antibody may also promote phagocytosis or may possibly cause direct lysis of viruses with lipid envelopes.

In general, secretory IgA is important in neutralizing viruses that enter through the mucosa, while circulating antibodies (IgM & IgG) are effective against viruses which pass through the blood stream before reaching their target cells.

- **T cell response**

- Cytotoxic T cells:

*The **main mechanism of specific immunity against established viral infections is killing of infected cells by Tc cells.** This mechanism is effective against viruses before being released from the infected cell. It is particularly important against those viruses which tend to remain for long periods inside host cells without being released and which are, therefore, inaccessible to antibodies.*

- Helper T cells:

These contribute by secretion of cytokines. The Th1 cytokines IL-2 and IFN- $\gamma$  are most important in this respect:

1. IL-2 promotes proliferation and activation of Tc cells and also activates NK cells (LAK cells).
2. IFN- $\gamma$  activates NK cells.

## IV. Immunity to Fungi

### A- Innate Immunity

- The neutrophil is the most important cell of the innate immune system in combating fungi. Neutrophils liberate fungicidal substances and phagocytose fungi as well. People with neutropaenia are extremely sensitive to fungal infections.
- Macrophages can also combat fungal infections.

### B- Specific Immunity

Most pathogenic fungi behave like intracellular bacteria and specific immunity to them is quite similar.

- **Humoral immune response**

Antibodies are often produced against fungi, but do not appear to be useful in protection.

- **T cell response**

Cell mediated immunity is the **major** defence mechanism against fungal infections. It acts in the same way as for combating intracellular bacterial infections. Similarly, the Th1 response is protective and the Th2 response is harmful to the host.

## How Organisms Evade the Immune Response:

As mentioned before, the survival of pathogenic microbes in a host depends on the ability of these microbes to evade or resist the body's immune mechanisms. Following are some examples:



1. Some extracellular bacteria, such as *Streptococcus pneumoniae*, evade phagocytosis by producing a polysaccharide capsule.
2. Intracellular organisms are capable of surviving inside macrophages after they are phagocytosed. *Mycobacterium tuberculosis*, for example, has sulpholipids that inhibit phagolysosome formation. They are thus able to survive and multiply within the phagosomes without being destroyed by lysosomal enzymes.
3. Small RNA viruses, such as influenza virus and HIV tend to mutate frequently, and antigens belonging to them change continually, thus evading immunological memory.
4. Large DNA viruses, such as herpes virus, evade the immune system by downregulating the expression of MHC I molecules on the cells they infect. The result is inability of cytotoxic T cells to recognize and kill such infected cell

**MCQs:**

- 1- **Humoral immunity may combat extracellular bacteria by any of the following EXCEPT:**
  - a- Agglutination
  - b- Neutralization of bacterial toxins
  - c- Opsonization
  - d- Complement activation
  - e- ADCC
- 2- **The main immune response against intracellular bacteria is:**
  - a- Type I interferon
  - b- ADCC by NK cells
  - c- Macrophages activated by Th1 cells
  - d- Cytotoxic killing by Tc cells
  - e- Neutralization by specific antibodies
- 3- **Innate immunity to viruses includes:**
  - a- Type I interferon
  - b- Cytotoxic T cells
  - c- Cytokines production by T helper cells
  - d- Neutralizing antibodies
  - e- Opsonizing antibodies
- 4- **The main specific immune defence mechanism against established viral infections is:**
  - a. Complement activation by alternative pathway
  - b. Antibody-mediated immune response
  - c. Cytotoxic T cell-mediated immune response
  - d. Helper T cell-mediated immune response
  - e. Killing by NK cells
- 5- **The most important cells involved in innate immunity to fungi are:**
  - a. Eosinophils
  - b. Helper T lymphocytes
  - c. Neutrophils
  - d. Cytotoxic T lymphocytes
  - e. Macrophages

## TUMOUR IMMUNOLOGY

### **ILOs:**

**By the end of this chapter the student should be able to:**

- Define immune-surveillance
- Show the evidence of immuno-surveillance
- Differentiate between tumour specific antigens (TSA) & tumour associated antigens (TAA)
- Represent the effector immune mechanisms involved in tumour immunology
- Explain the mechanisms by which CTLs recognize and kill tumour cells
- Explain the mechanisms by which NK cells recognize and kill tumour cells
- Recognize the role of cytokines in destruction of tumour cells
- Summarize the methods by which tumours evade the immune system
- Illustrate the therapeutic approaches that aim to enhance the immune response against malignant cells

Tumour cells may develop surface molecules which are antigenically new (**tumour antigens**). They can be recognized by the immune system as foreign.

**Immuno-surveillance** is the ability of the immune system to prevent the development of tumours through early recognition and destruction of tumour cells.

### **Evidence of Immune Response to Tumours** (Evidence of Immuno-Surveillance)

1. Immunosuppressed patients (e.g. AIDS patients) develop tumours frequently.
2. Very young and very old people have an increased incidence of tumours. These members of the population often have less effective immune response.
3. Antibodies and T lymphocytes against tumour antigens have been detected in patients with tumours.
4. Animals can be specifically immunized against various types of tumours.

## Tumour Antigens

Tumour antigens can be very useful **tumour markers** in the diagnosis and follow-up of various tumours.

Two groups of tumour antigens have been described (Fig. 35):

### 1. Tumour-specific antigens (TSAs):

These antigens are not found on normal somatic cells. They have been identified on:

- Tumours induced by viral transformation, such as those caused by human papilloma virus, hepatitis B virus and Epstein Barr virus.
- Tumours resulting from genetic mutations due to exposure to carcinogenic chemicals, x-ray, ... etc.

### 2. Tumour-associated antigens (TAAs):

These antigens are not unique to tumour cells, rather it is their expression on tumour cells that is altered. They may be:

- Normal self proteins found in **excessive amounts** on tumour cells compared to normal cells. For example, a certain receptor called HER2 is found on normal cells in very low amounts, but is overexpressed on some breast tumours.
- Proteins normally expressed only on embryonic cells but which reappear on tumour cells in adults. They are therefore called **oncofoetal antigens** (e.g.  $\alpha$ -foetoprotein and carcinoembryonic antigen).

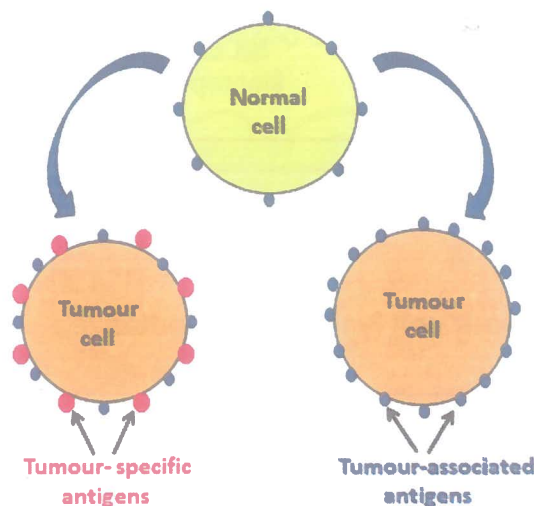


Fig. (35): Tumour antigens

## Effector Mechanisms Involved in Tumour Immunology

Both innate and specific immunity play a role in combating tumours and can affect the growth and progression of a tumour.

### A. Innate Immunity

The first line of immune defence is the innate immune system. Innate immune mechanisms are not specific to particular tumour antigens but recognize general characteristics of tumour cells.



- Monocytes/macrophages play an important role through:
  1. Antigen presentation
  2. Cytokine secretion e.g. TNF- $\alpha$
  3. Direct cytotoxicity to tumour cells
  4. Killing of tumour cells by ADCC
- NK cells recognize tumour cells and kill them either directly or through ADCC.

## B. Specific Immunity

### • Humoral Immune response

Tumour-specific antibodies may cause tumour cell lysis by (Fig. 36):

1. Fixing complement to the tumour cell membrane, resulting in the formation of membrane attack complex and consequent lysis of tumour cells
2. Antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells and possibly other cells such as macrophages

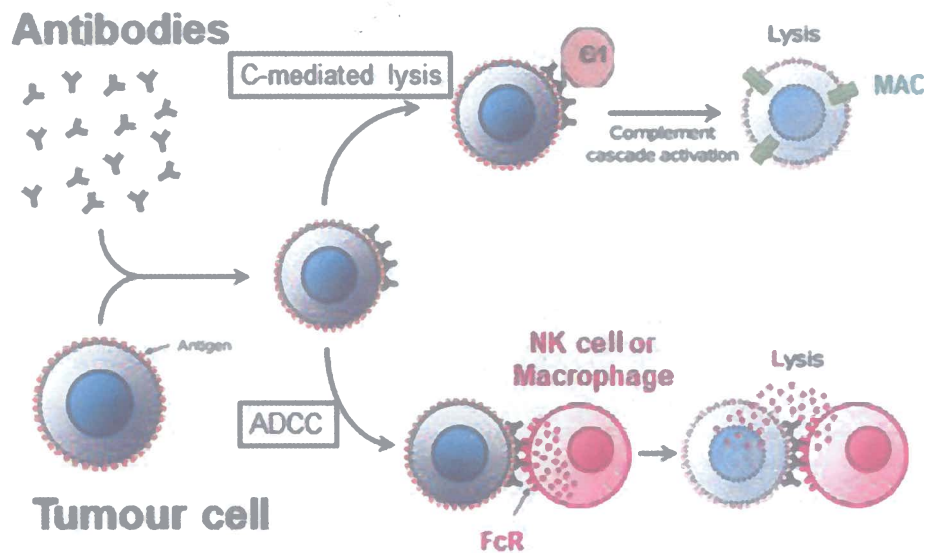


Fig. (36): Tumour cell lysis by antibodies

### • T cell response

- 1- Cytotoxic T cells recognize antigens in association with MHC I molecules on tumour cells and kill them (Fig. 37).

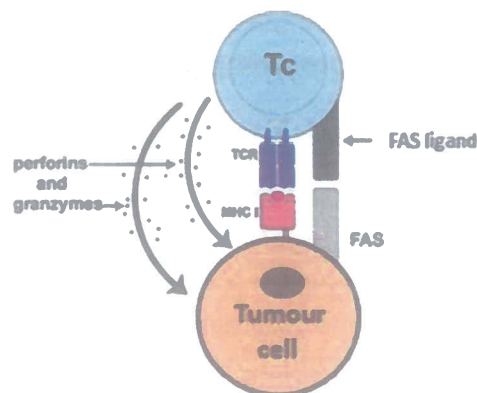


Fig. (37): Anti-tumour action of Tc cells

2- Helper T cells are activated by shed tumour antigens which become internalized and presented on the surface of APCs in association with MHCII.

Some important cytokines released by Th1 cells are:

- IL-2 which activates Tc cells, NK cells and B cells
- IFN- $\gamma$  which activates macrophages and NK cells
- TNF which is directly toxic to tumour cells

*Note: Tumour-infiltrating lymphocytes (TILs) are T cells (mainly Tc) that are present within tumours.*

### Tumour Evasion (Escape) of the Immune Response

A number of mechanisms have been suggested for the escape of tumours from immuno-surveillance: (Fig. 38)

1. The host may be immunocompromised.
2. Tumours may be localized at sites inaccessible to the immune system e.g. CNS.
3. Certain tumours (virally-induced) express reduced levels of MHC I molecules.
4. Some viruses block expression of co-stimulatory molecules (e.g. B7) by antigen-presenting cells.
5. Factors related to tumour antigens:
  - Some tumours lack antigens that can stimulate the immune response.
  - Some tumour antigens cannot be processed and presented with MHC.
  - Amount of antigen may be too small to stimulate the immune system.
  - Some tumours may shed their antigens which block antibodies and T cells from reacting with the tumour cells.
  - Tumour antigens may become masked by a fibrin coat.

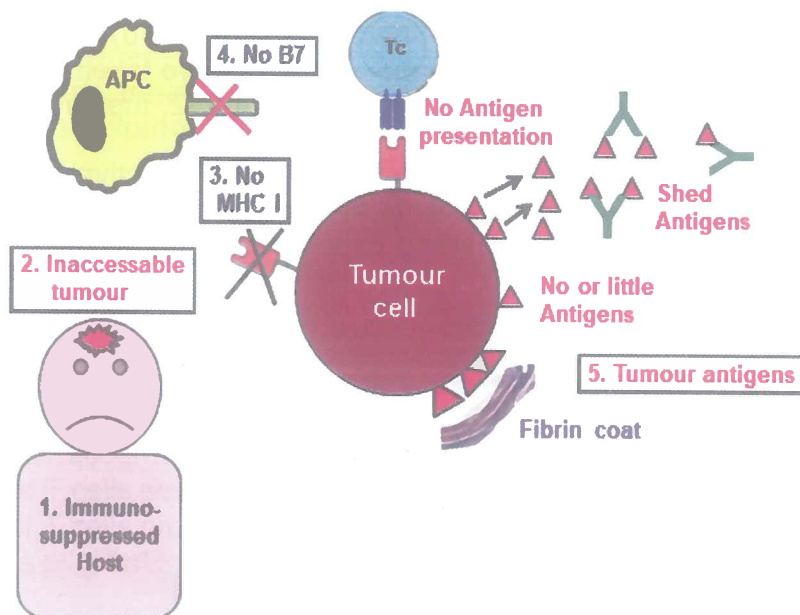


Fig. (38): Tumour Evasion

### Approaches to Tumour Immunotherapy

These approaches aim to enhance the natural immune responses that the body mounts against malignant cells.

### I- Monoclonal antibodies (mAbs):

Monoclonal antibodies directed against tumour antigens may be used either alone (naked) or coupled (conjugated) to radioisotopes, cytotoxic drugs, toxins (e.g. diphtheria toxin) or cytokines. Coupling has the advantage of delivering high doses of radioactivity or cytotoxic drugs directly to the site of the tumour (**magic bullet therapy** (Fig. 39).

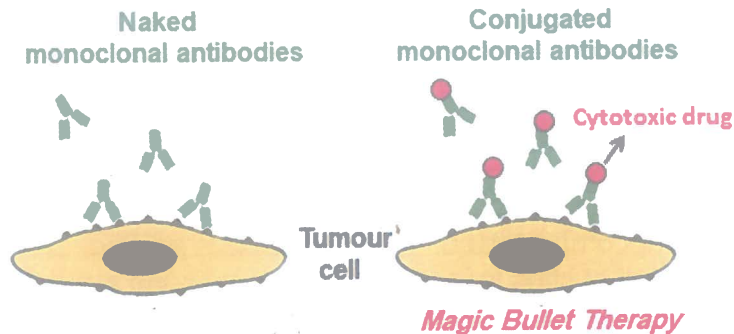


Fig. (39): Monoclonal antibodies against tumours

Examples of monoclonal antibodies used successfully:

#### a- Anti-CD20:

- CD20 is expressed on B-cells.
- Anti-CD20 is used either naked or conjugated with radioactive iodine in the treatment of B-cell lymphomas.

#### b- Trastuzumab (Herceptin®):

- It is an antibody against the **HER2 protein**.
- It is used to treat breast cancers that express large amounts of this protein.

### II- Adoptive cell therapy (infusion of immune effector cells): (Fig. 40)

- The patient's own tumour-infiltrating lymphocytes (TILs) are taken directly from tumour biopsies, expanded *in vitro* in the presence of IL-2, then injected back into the patient.
- NK cells from the patient are treated with IL-2 in order to activate them. The result is **lymphokine-activated killer (LAK) cells** that are much more effective than ordinary NK cells at killing tumour cells. These cells are then injected into the patient.

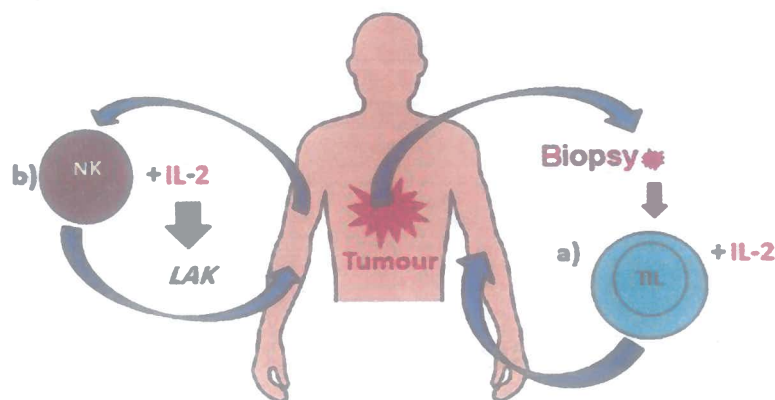


Fig. (40): Adoptive cell therapy



**III- Anti-tumour vaccines:****a- Tumour cell vaccines:**

Irradiated tumour cells (from the patient himself or from other patients) are mixed with bacterial adjuvant e.g., BCG and injected into the patient in order to stimulate a good immune response.

**b- Tumour antigen vaccines:**

Synthetic peptides can be used as anti-tumour vaccines.

**N.B.:** Active immunization against oncogenic viruses can prevent the development of certain tumours. Two excellent examples are:

- The successful mass immunization against hepatitis B virus which is already decreasing the incidence of primary hepatoma in endemic areas
- Immunization against human papillomavirus which causes cervical cancer.

**IV- Nonspecific Immunotherapy:**

**a- Interferons (IFNs)** have anti-tumour activity in leukaemia and AIDS-associated Kaposi's sarcoma.

**b- Bacterial adjuvants** e.g. BCG, may be used with or without tumour antigens, to treat a wide variety of cancers.

**MCQs:****1- Tumour antigens:**

- a- Are always specific for a certain tumour
- b- May be shed from the tumour surface and thus enhance tumour rejection
- c- May be of viral origin
- d- Are never found on normal cells
- e- Are recognized by cytotoxic T cells when carried on MHC II molecules

**2- Cells of the innate immune system that have an important role in destruction of tumour cells are:**

- a- B cells
- b- T helper cells
- c- T cytotoxic cells
- d- Neutrophils
- e- NK cells

**3- In the immune response to tumours:**

- a- T cells are the main cells responsible for anti-tumour immunity.
- b- Tc cells recognize antigens associated with MHC II molecules on tumour cells
- c- Helper T cells secrete cytokines which activate mast cells.
- d- Helper T cells produce histamine which is directly toxic to tumour cells.
- e- B cells play an important role in presenting tumour antigens to mast cells

**4- The following mechanisms may contribute to tumour evasion EXCEPT:**

- a- Lack of expression of MHC II on tumour cells
- b- Lack of antigens on tumour cells that can stimulate the immune response
- c- Blocking the expression of co-stimulatory molecules
- d- Localization of tumour in an inaccessible site
- e- Shedding of tumour antigens that block antibodies and T cells

## HYPERSENSITIVITY REACTIONS

### **ILOs:**

**By the end of this chapter the student should be able to:**

- Classify types of hypersensitivity
- Explain the immuno-pathogenic mechanisms of type I (immediate) hypersensitivity reaction
- Recognize the clinical conditions, laboratory diagnosis and therapeutic measures of type I (immediate) hypersensitivity reaction
- Explain the immuno-pathogenic mechanisms of type II (cytotoxic) hypersensitivity reaction
- Recognize the clinical conditions and laboratory diagnosis of type II (cytotoxic) hypersensitivity reaction
- Explain the immuno-pathogenic mechanisms of type III (immune-complex) hypersensitivity reaction
- Recognize the clinical conditions and therapeutic measures of type III (immune-complex) hypersensitivity reaction
- Explain the immuno-pathogenic mechanisms of type IV (cell-mediated) hypersensitivity reaction
- Recognize the clinical conditions of type IV (cell-mediated) hypersensitivity reaction

In the previous chapters, we discussed how immunological mechanisms can operate to the benefit of the host in overcoming microbial infections and in protecting from tumours. It is the fighting army for the host to preserve his integrity. However, there is no war without victims. So, it might be expected that some forms of tissue damage may arise from exaggerated or inappropriate immunological responses.

The mechanisms by which immunological tissue damage occurs are classified into 4 categories:

1. Anaphylactic reaction
2. Cytotoxic (cytolytic) reaction
3. Immune-complex-mediated reaction
4. Cell-mediated reaction

These reactions may occur in cases of **hypersensitivity, auto-immune disease or graft rejection**.

## HYPERSENSITIVITY

Hypersensitivity reactions are inappropriate immune responses to certain antigens causing tissue damage. Typically, hypersensitivity reactions occur following re-exposure of an individual to the antigen; the first contact induces an immune response (sensitization), while subsequent exposure produces a reaction that leads to tissue damage.

Hypersensitivity reactions have been classified into 4 types that correspond to the 4 mechanisms of tissue damage.

### TYPE I: IMMEDIATE (ANAPHYLACTIC) HYPERSENSITIVITY REACTIONS

Type I hypersensitivity reactions are exaggerated immune responses to harmless environmental antigens (allergens). They occur almost exclusively in hypersensitivity (not during autoimmune disease or graft rejection). They are mediated by IgE.

**Mechanism:** (Fig. 41)

#### A- Sensitization

- In normal individuals, antigenic stimulation results in the production of extremely low levels of IgE. However, certain individuals have a genetic predisposition to allergy. They respond to certain antigens (allergens) by forming large amounts of IgE.
- The production of IgE is under the control of Th2 cells. These cells produce IL-4, which is the critical stimulus for inducing class switching from IgM to IgE, resulting in the production of large amounts of allergen-specific IgE.
- The IgE molecules bind to Fc receptors on mast cells (and basophils). Thus, first exposure to an allergen results in sensitization of the mast cells (and basophils).

#### B- Degranulation of mast cells

Upon subsequent exposure to the same allergen, the IgE molecules on the sensitized mast cells become cross-linked by the allergen. This induces degranulation of mast cells and the release of 2 kinds of mediators:

**a- Preformed mediators:** Histamine and platelet activating factor (PAF) are the mediators of symptoms seen during the **early phase**, which occurs within 15-20 minutes of exposure to the allergen.

**b- Newly formed mediators:** The leukotrienes and prostaglandins, which take several hours to be synthesized, cause the symptoms seen during the **late phase**. The late phase typically occurs 5-6 hours after allergen contact. The symptoms of the late phase are the same as those of the early phase, but persist longer. This late phase involves the recruitment of other effector cells, notably Th2 lymphocytes, eosinophils and basophils which contribute significantly to the inflammatory response and tissue damage.



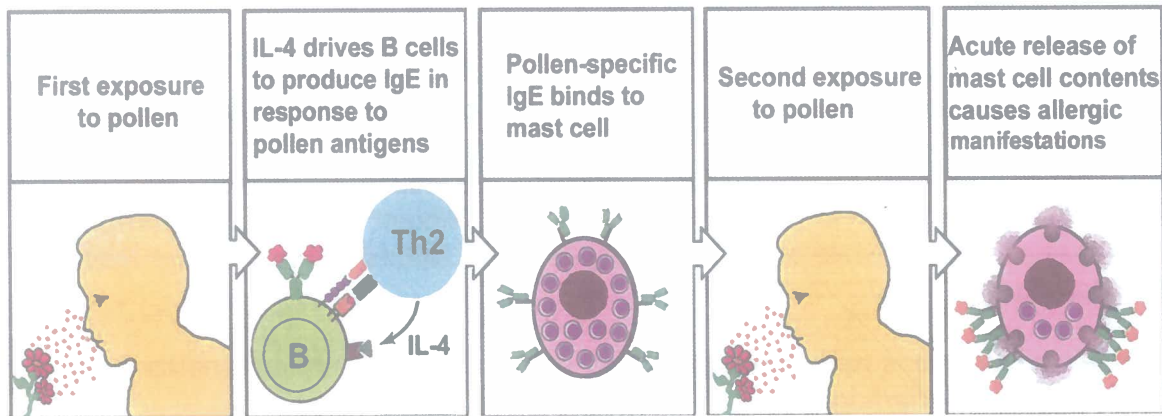


Fig. (41): Mechanism of type I hypersensitivity reactions

## Clinical Forms

### 1. Systemic anaphylaxis:

This is the most severe form of type I hypersensitivity, in which severe bronchoconstriction and hypotension (shock) can be life-threatening.

Anaphylaxis may occur due to previous sensitization by foreign antigens, e.g. antitoxic serum prepared in animals for treatment of tetanus and diphtheria. It also occurs with penicillin and many other drugs that act as haptens and attach to body proteins.

### 2. Localized anaphylaxis (Atopy)

- In this form, the symptoms are localized in one organ or system.
- The allergens to which the individual is sensitized are widely distributed in nature. They are classified as:
  - **inhalants**, e.g. pollens, house dust, moulds
  - **contactants**, e.g. wool, animal fur, nylon
  - **ingestants** e.g. many food stuffs.
- Symptoms vary widely from one individual to another depending on the site of antigen-antibody reaction. Thus, it may take the form of bronchial asthma, urticaria, conjunctivitis, gastrointestinal symptoms or allergic rhinitis.

## Diagnosis

1. Detection of the antigen to which the individual is sensitive by skin test in which extracts of various antigens are introduced into the skin of the patient. Positive cases show a wheal and flare reaction that appears within 15-25 minutes at the site of the antigen to which the individual is allergic (Fig. 42)
2. Measurement of levels of total IgE and IgE specific for a particular allergen.

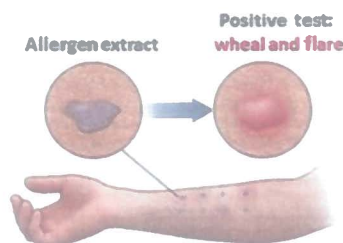


Fig. (42): Allergy skin test

## Therapeutic Measures

### I. Management of anaphylactic shock

Anaphylactic shock is an emergency which must be dealt with immediately by administration of adrenaline and corticosteroids, together with oxygen inhalation.

### II. Management of atopy

1. **Avoidance** of the responsible allergen
2. **Specific immunotherapy:** This involves administration of gradually increasing doses of the responsible allergen to the patient over time. The aim is shifting the immune response from Th2 to Th1 response, leading to down-regulation of IgE production (desensitization).
3. **Drugs** that block the release of the mediators or counteract their effects, e.g. antihistaminics, corticosteroids and anti-leukotrienes
4. **Monoclonal anti-IgE antibodies:** These antibodies attach to the Fc portion of IgE and prevent it from attaching to mast cells.

## TYPE II: CYTOLYTIC (CYTOTOXIC) HYPERSENSITIVITY REACTIONS

This type of hypersensitivity is mediated by IgG or IgM antibodies reacting with cell-bound antigen (surface antigen). This antigen may be originally present on the cell membrane or an antigen (or hapten) that became attached to it. This cell may then undergo one of the following:

1. It may be destroyed by one of the following destructive processes (Fig. 43):
  - Lysis of target cells via activation of complement
  - Opsonization of target cells with or without complement activation
  - Lysis of target cells by antibody-dependent cell-mediated cytotoxicity (ADCC)
2. It may undergo functional alteration. This occurs when the antibody attaches to a receptor on the cell, leading to stimulation or blocking of that receptor.

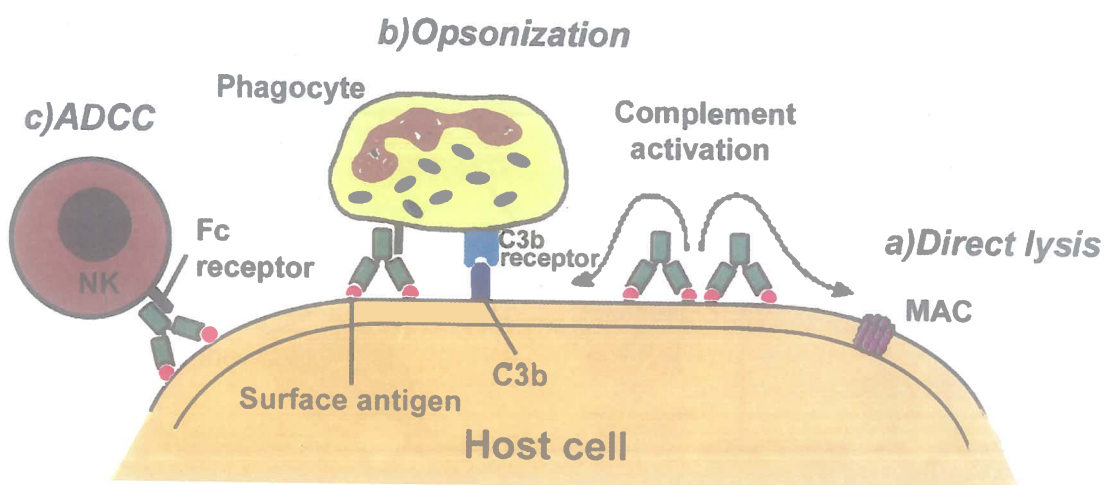


Fig. (43): Mechanism of cell destruction by type II hypersensitivity reactions

## Examples of Cytolytic (Cytotoxic) Reactions

1. **Blood group (ABO or Rh) incompatibility**
2. **Autoimmune diseases:** autoantibodies may develop against an individual's own cells resulting in various types of autoimmune diseases.
3. **Drug reaction:** a drug may become attached to a cell surface. Antibodies to the drug reacting at the cell surface lead to destruction of the cell in presence of complement.
4. **Graft rejection**

## TYPE III: IMMUNE-COMPLEX HYPERSENSITIVITY REACTIONS

When antibodies combine with their specific antigen, immune complexes are formed. Normally, they are effectively removed by the reticuloendothelial system (macrophages). Occasionally, small soluble immune complexes are formed, especially when there is antigen excess. These complexes, being very small, may escape phagocytosis and become deposited in tissues (especially in joints and kidneys) leading to tissue damage.

### Mechanism of Tissue Damage: (Fig. 44)

1. The first step is the formation of soluble immune complexes that are formed of antigen and IgG or IgM.
2. These immune complexes penetrate the endothelium of blood vessel walls and become deposited on the vascular basement membrane.
3. Complement is activated and C3a and C5a (anaphylatoxins) are released:
  - a. These anaphylatoxins react with receptors on mast cells and basophils, causing release of vasoactive amines (e.g. histamine), which increase vascular permeability.
  - b. C5a is also chemotactic for neutrophils which infiltrate the area. In an attempt to engulf the immune complexes, these phagocytic cells degranulate and release lysosomal enzymes that destroy the basement membrane.
4. Platelets are aggregated with two consequences; they release vasoactive amines and form microthrombi which cause local ischaemia and further tissue damage.

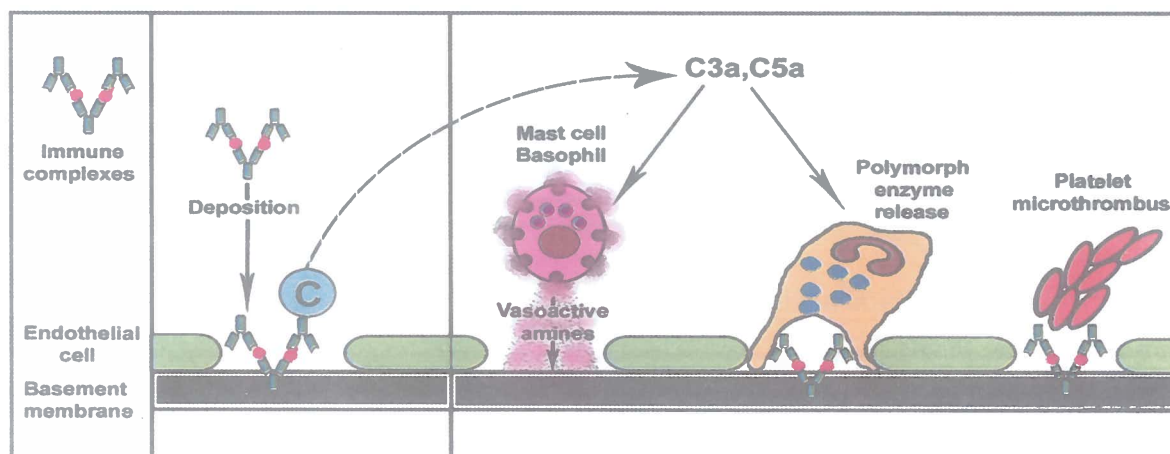


Fig. (44): Mechanism of type III hypersensitivity reactions



## Examples of Immune-Complex-Mediated Reactions

### 1. Serum Sickness

This syndrome is considered to be a systemic immune-complex disease. It occurs following injection of a large amount of foreign serum (e.g. horse antitetanic or antidiphtheritic serum) or drugs (e.g. penicillin) into humans. The antigen is slowly cleared from the circulation, while antibody production begins. These antibodies react with remnants of antigens still present. Immune complexes are formed, which may become deposited at various sites causing the manifestations of the disease. Typical serum sickness results in fever, urticaria, arthralgia, lymphadenopathy, and splenomegaly, which develop a few days to 2 weeks after injection of the foreign serum or drug.

### 2. Arthus reaction

This is a form of local immune complex disease due to repeated subcutaneous injection of a low dose of a foreign antigen, e.g. insulin and rabies vaccine. The reaction occurs at the site of antigen injection. Immune complexes are deposited in the walls of blood vessels, complement is activated, and the previously mentioned inflammatory response is initiated resulting in local erythema, oedema and necrosis.

### 3. Post-streptococcal glomerulonephritis

### 4. Viral infections: e.g. hepatitis B

### 5. Autoimmune diseases: e.g. rheumatoid arthritis and systemic lupus erythematosus (SLE)

### 6. Hypersensitivity pneumonitis: Some inhaled antigens, e.g. dust, pollen and fungal spores provoke IgG rather than IgE antibody responses. Immune complexes are formed in the alveolar wall leading to pneumonitis.

## Therapeutic Measures

### 1. Reduction of inflammation by means of antihistaminics and corticosteroids

### 2. Suppression of the immune response by means of corticosteroids and immunosuppressive drugs

### 3. Removal of offending complexes via plasmapheresis (exchanging the patient's plasma with normal plasma, thereby removing the immune complexes)

## TYPE IV: CELL MEDIATED (DELAYED) HYPERSENSITIVITY REACTIONS

It is an exaggerated cell mediated immune response that damages host cells. The main cell involved is the activated T lymphocyte (mainly Th1). Antibody and histamine play no role in this type. The response is delayed (starts hours or days after contact with the antigen).

### Mechanism: (Fig. 45)

Th1 cells recognize antigens and release pro-inflammatory cytokines. These act on the vascular endothelium leading to increased vascular permeability and leakage of fluid into the tissues. They also attract and activate monocytes and macrophages as well as more T cells. These inflammatory reactions lead to local tissue damage. Cytotoxic T cells may also play a role.

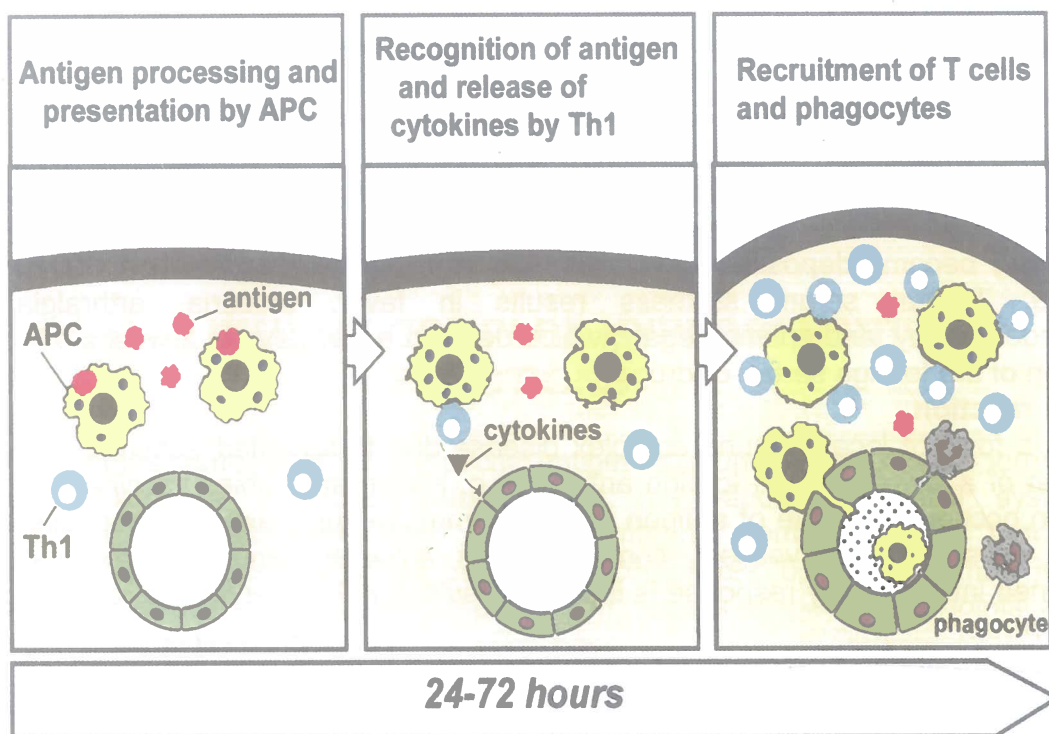


Fig. (45): Mechanism of type IV hypersensitivity reactions

### Examples of Cell-Mediated Reactions

1. The tuberculin skin test reaction is the classic clinical example of delayed hypersensitivity:

- A small amount of the antigen, purified protein derivative (PPD) of tubercle bacilli, is injected intradermally into a person's forearm.
- In a person previously exposed to tubercle bacilli (sensitized), a positive reaction occurs as an indurated area, 48-72 hours after injection. This induration is due to accumulation of macrophages and lymphocytes.

### 2. Contact dermatitis

- The manifestations of cell mediated hypersensitivity occur after sensitization with chemicals, plant materials, topically applied drugs and some cosmetics.
- In all cases, the small molecules acting as haptens enter the skin, attach to body proteins, which are taken up by APCs in the skin (Langerhan's cells) to be presented to T cells.
- Upon later skin contact with the offending agent, the sensitized person
- develops erythema, itching, vesicles or eczema of the skin within 12-48 hours.

### 3. Granuloma formation (Fig. 46)

- Granulomatous inflammation is a form of delayed type hypersensitivity that occurs in response to persistence of antigen within macrophages. This antigen may be an infectious agent such as *M. tuberculosis* and some fungi, or a particulate non-degradable antigen, such as silica.

- The granuloma consists of a cluster of activated macrophages (termed epithelioid cells), which often fuse into multi-nucleated giant cells. The macrophages are surrounded by a collar of T lymphocytes (mainly Th cells). The core of the lesion may undergo necrosis.
- Granuloma formation occurs under the influence of cytokines produced by the activated Th cells.

#### 4. Autoimmune diseases

#### 5. Graft rejection

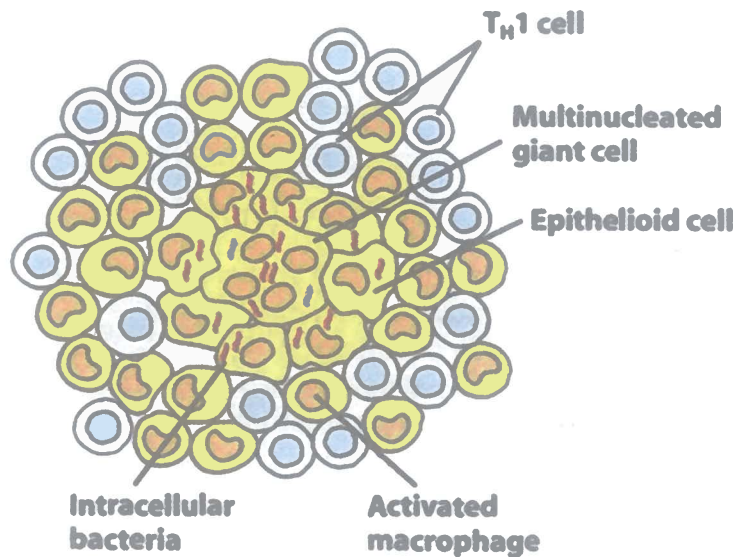


Fig. (46): Granuloma

#### MCQs:

- 1- **On exposure to allergen, sensitized mast cells:**
  - a. Release preformed mediators after 12 hours
  - b. Undergo degranulation because of cross-linking of IgD on their surfaces
  - c. Bind IgE to their Fc receptors
  - d. Release IL-2 for further IgE production
  - e. Release histamine and form other mediators
- 2- **Desensitization performed for the treatment of atopy:**
  - a- Increases IL-4 production
  - b- Causes shift of Th1 to Th2 response
  - c- Causes shift of Th2 to Th1 response
  - d- Blocks release of mediators from sensitized mast cell
  - e- Involves injecting patients with gradually decreasing doses of the allergen



- 3- Adverse effects following incompatible blood transfusion occur as a result of:**
- a- Anaphylactic reactions
  - b- Cytolytic hypersensitivity reactions
  - c- Immune complex hypersensitivity reactions
  - d- Cell mediated hypersensitivity reactions
  - e- None of the above
- 4- Immune complex-mediated reactions include:**
- a- Post-streptococcal glomerulonephritis
  - b- Asthma
  - c- Anaphylaxis
  - d- Contact dermatitis
  - e- Allergic rhinitis
- 5- Serum sickness:**
- a- Is a systemic form of type II hypersensitivity reactions
  - b- Is a local form of immune complex disease
  - c- Is mediated by IgM or IgG
  - d- May occur as a result of cell mediated immune reaction
  - e- May be treated by administration of interferon
- 6- The main cells involved in type IV hypersensitivity are:**
- a- Cytotoxic T cells
  - b- Th1 cells
  - c- Th2 cells
  - d- B cells
  - e- NK cells

## TRANSPLANTATION IMMUNOLOGY

### **ILOs:**

**By the end of this chapter the student should be able to:**

- Identify the types of MHC antigens, their genetic origin, structure, function and role as transplantation antigens
- Show the significance of MHC molecules
- Define the different types of grafts
- List the antigens responsible for graft rejection
- Compare between the types of transplant rejection
- Explain the immune mechanisms of transplant rejection
- Define graft-versus-host disease and outline its preventive measures
- Represent the preventive measures against graft rejection
- Define tissue-typing and give an outline of different techniques used for tissue-typing

### **The Major Histocompatibility Complex (MHC) (Fig.47)**

- This is a group of genes which is found on the short arm of chromosome 6.
- MHC genes are divided into 3 major classes, class I, class II and class III :
  - Class I and class II genes code for MHC molecules, which are antigens expressed on cell surface membranes.
  - Class III genes code for a number of complement components and play no role in transplantation. They happen to be grouped together in a region between class I and class II genes.
- MHC antigens are also called HLA (human leucocyte antigens) because they were first discovered on the surface of human leucocytes.
- There are three class I loci (HLA-A, B and C). Each locus is highly polymorphic i.e. a single HLA locus contains one of many possible alleles. The various possible alleles are given consecutive numbers, e.g. HLA-A1, HLA-A2, etc. Thus, most individuals will have genes for six different class I molecules, all of which will present at the cell surface.
- Class II molecules are encoded by three principal loci (HLA-DP, -DQ and -DR), which also show polymorphism.
- They may act as immunogens when introduced to a genetically distinct individual.
- MHC molecules are co-dominantly expressed from both maternal and paternal chromosomes.

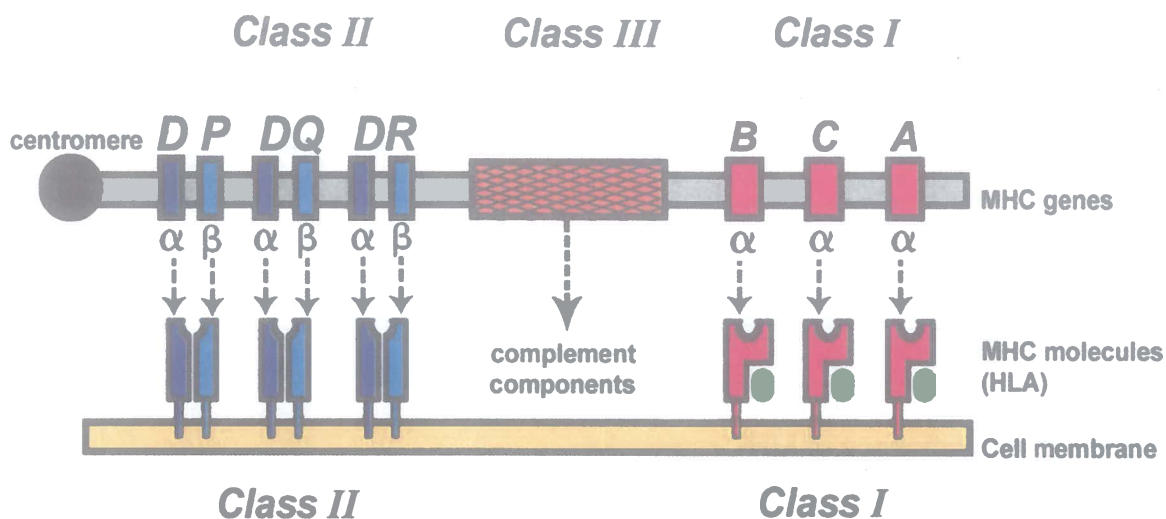


Fig. (47): The major histocompatibility complex (MHC)

### Structure and distribution of MHC I and MHC II molecules (Fig. 48)

- Class I MHC molecules are found on the surface of all nucleated cells. Each molecule is composed of two polypeptide chains: heavy or  $\alpha$  chain and  $\beta$ 2-microglobulin chain. The MHC class I genes code for the heavy chains ( $\alpha$  chains), while  $\beta$ 2-microglobulin chain is coded for elsewhere in the genome.
- Class II MHC molecules are mainly found on the surface of the professional antigen presenting cells (APCs). The class II MHC molecule is a dimer composed of two glycoprotein chains:  $\alpha$  chain and  $\beta$  chain. MHC class II genes code for both  $\alpha$  and  $\beta$  chains.

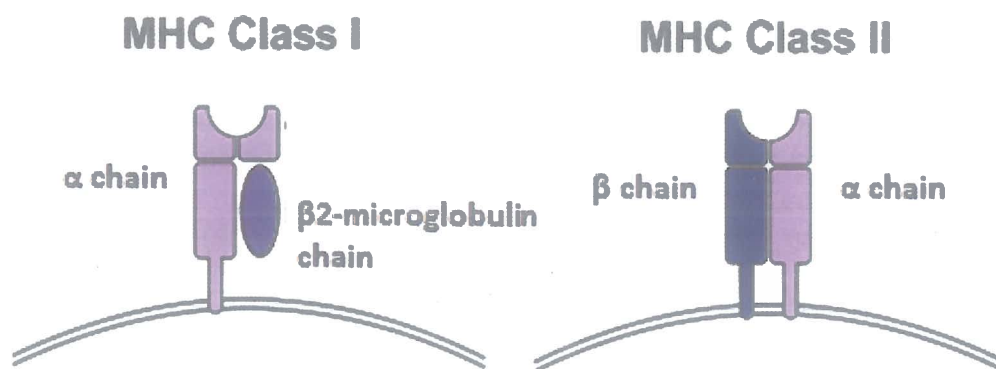


Fig. (48): Structure of MHC

\* Alleles: variants of a single genetic locus.



### Significance of MHC molecules

1. MHC I and II molecules present peptides to naïve T cells, leading to their activation.
2. MHC I molecules enable effector Tc cells to recognize target cells and MHC II molecules on macrophages and B cells enable effector Th cells to recognize and help them.
3. Graft rejection is a consequence of mismatching of donor and recipient MHC molecules.
4. Certain MHC alleles affect the susceptibility of an individual to some inflammatory or autoimmune diseases.
5. They are used in forensic medicine, e.g. disputed paternity.

### The Minor Histocompatibility Complex

This is a group of genes which code for antigens that cause weaker rejection responses than MHC antigens.

## TRANSPLANTATION

### Types of Grafts (Fig. 49)

1. **Autografts:** are grafts from one part of the body to another. They are not foreign and, therefore, do not elicit rejection.
2. **Isografts:** are grafts between genetically identical individuals (identical twins). As they do not express antigens foreign to the recipient, they are not rejected.
3. **Allografts:** are the common clinical transplants, where one person donates an organ to a genetically different individual of the same species. In this case, the cells of the allograft express antigens which are recognized as foreign by the recipient.
4. **Xenografts:** represent the maximal genetic disparity between members of different species and are generally rapidly rejected.

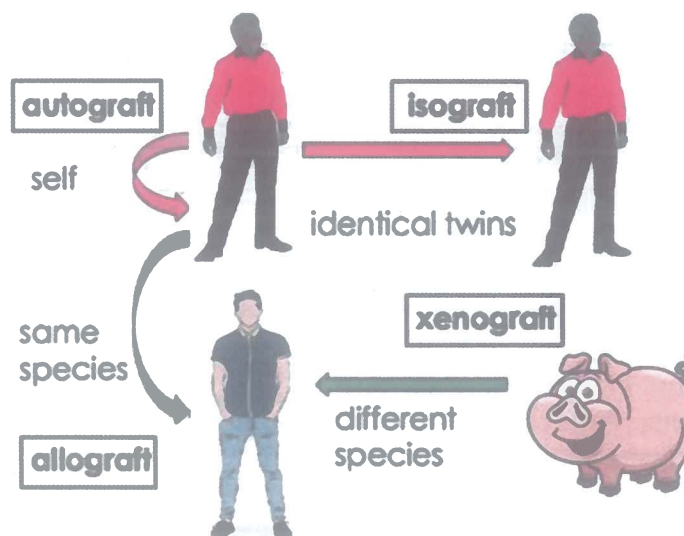


Fig. (49): Types of grafts

## Antigens Responsible for Immune Rejection

1. Blood group antigens of the ABO system
2. Major histocompatibility complex (MHC) antigens
3. Minor histocompatibility complex antigens

## Types and Mechanisms of Allograft Rejection

### I. Acute Rejection

- It is the **most common** type of rejection.
- It takes place when there is HLA incompatibility.
- It is a type IV (cell mediated) delayed hypersensitivity reaction.
- It takes **days or weeks** to develop because of the time taken for T cell activation, proliferation and migration into the donor graft.
- Both Tc and Th lymphocytes are involved in the process of rejection:
  - Tc cells cause direct destruction of transplanted cells.
  - Th cells release a number of cytokines which lead to activation of the immune response. IL-2 is the most important cytokine in cellular rejection and is required for activation of Tc cells. TNF- $\alpha$  and IFN- $\gamma$  act as macrophage activators.

### II. Hyperacute Rejection

- It is the **most dramatic** form of rejection.
- It is caused by preformed anti-donor antibodies binding to either ABO blood group or HLA class I antigens on the graft.
- It can be considered a type II hypersensitivity reaction.
- It takes place **within minutes** of transplantation, since the antibodies are preformed.
- Antibodies attach to the endothelial cells of the donor organ and fix complement, resulting in damage to the vascular endothelium and release of pro-inflammatory complement components C3a and C5a. Results include:
  - Haemorrhage
  - Platelet aggregation within the vessels and graft thrombosis
  - Lytic damage to cells of the transplant

### III. Chronic or Late Rejection

- It is the **slowest and least severe** type of rejection.
- It is probably due to low-grade cell-mediated rejection (type IV hypersensitivity reaction).
- It takes place **months or years** after transplantation, depending on the genetic disparity between donor and recipient, as well as the efficiency of immunosuppressive therapy.
- It has been noticed that chronic rejection is more prominent in patients who have chronic viral infections, especially with cytomegalovirus, or patients with pre-existing autoimmune disease.

## Graft-Versus-Host Disease (GVHD)

- This serious condition occurs when the graft reacts against the recipient's tissues, instead of the recipient reacting against the graft.

- It may follow bone-marrow transplantation in which immunologically competent T cells are transplanted into a genetically non-matching immunosuppressed recipient.
- It is characterized by fever, pancytopenia, rash and hepatosplenomegaly.
- Careful typing, use of immunosuppressive drugs and removal of mature T cells from the donor stem cells, reduce the risk of GVHD.

## Prevention of Rejection

### I. Selection of Donor:

#### 1- ABO matching:

It is the first step in choosing the donor, to avoid hyperacute rejection by preformed antibodies against the ABO blood group system.

#### 2- Histocompatibility testing:

- A perfectly matched donor and recipient would be genetically identical individuals (monozygotic twins). However, this situation is rare, and in all other cases there will be major and/or minor histocompatibility differences between donor and recipient.
- Since leucocytes carry all known HLA antigens, they can be used for tissue typing to identify HLA of both donor and recipient and to test for compatibility between them:

##### a) Tissue typing:

##### ❖ *Serological HLA typing (Lymphocytotoxicity test):* (Fig. 50)

- A purified suspension of lymphocytes is mixed with antisera against the different HLA antigens in the presence of complement.
- If the lymphocytes carry the appropriate antigen, they will combine with the antibodies in the serum and activate the complement, leading to cell death.
- Trypan blue dye is added to differentiate between dead and living cells.

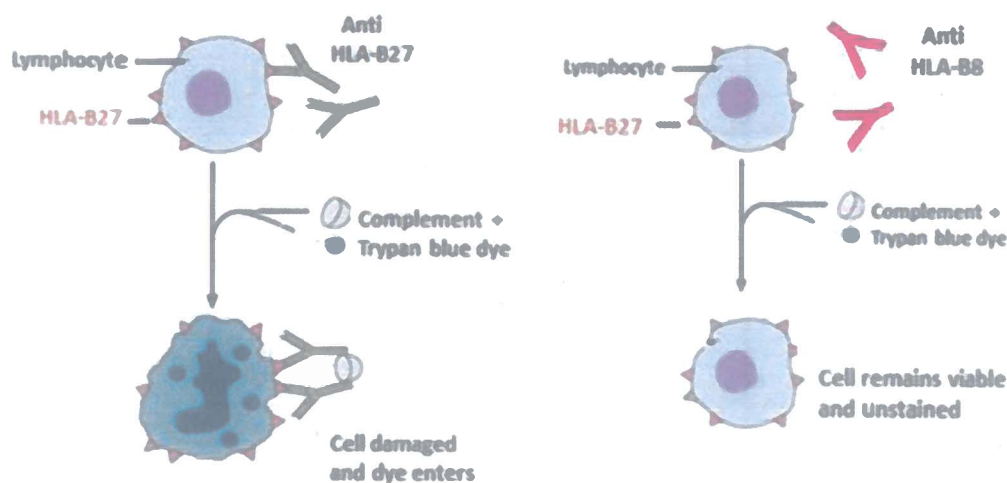


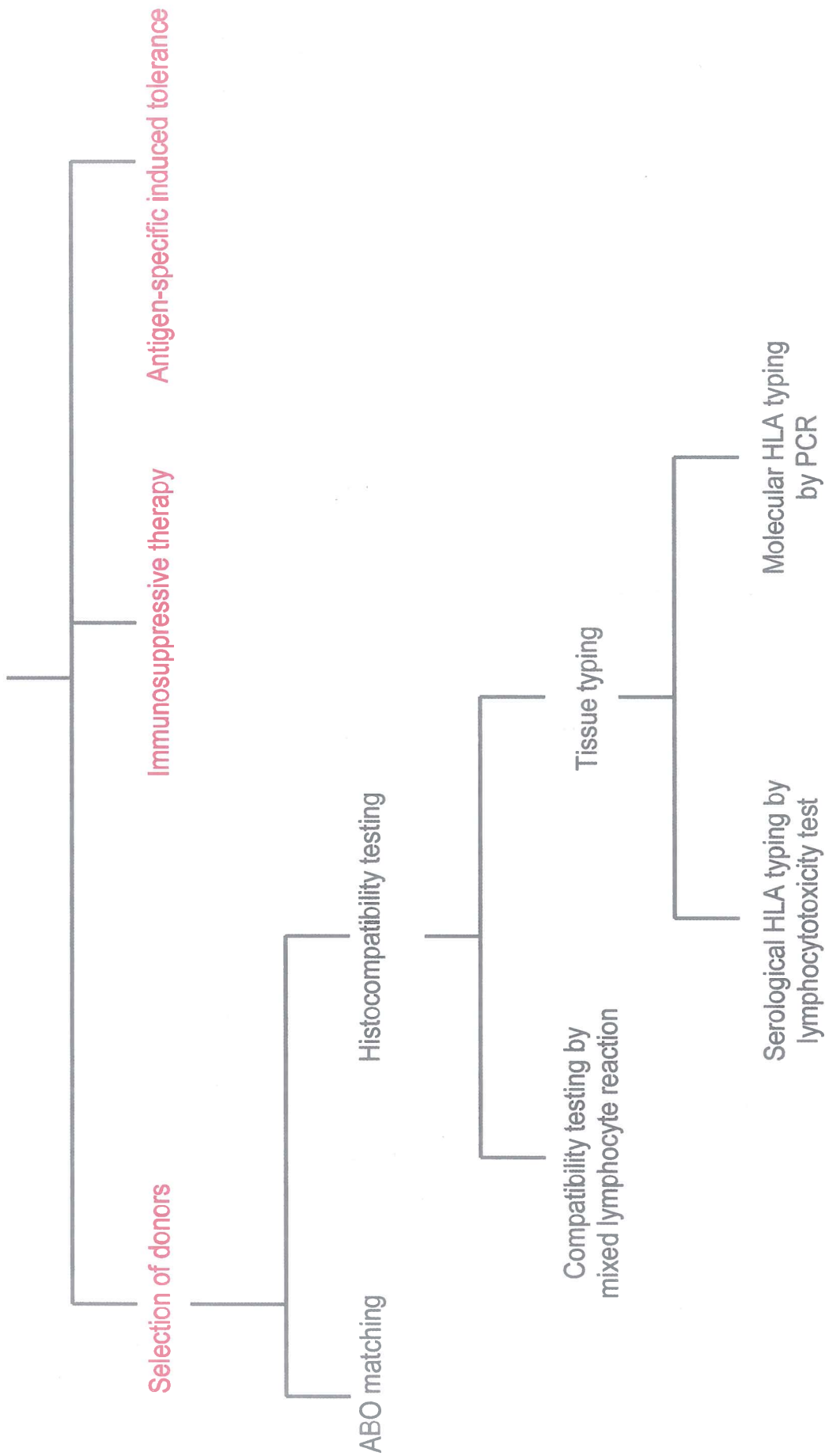
Fig. (50): Lymphocytotoxicity test

##### ❖ *Molecular HLA typing:*

Recently, sensitive and accurate typing has been achieved using PCR to identify HLA genes of donors and recipients.



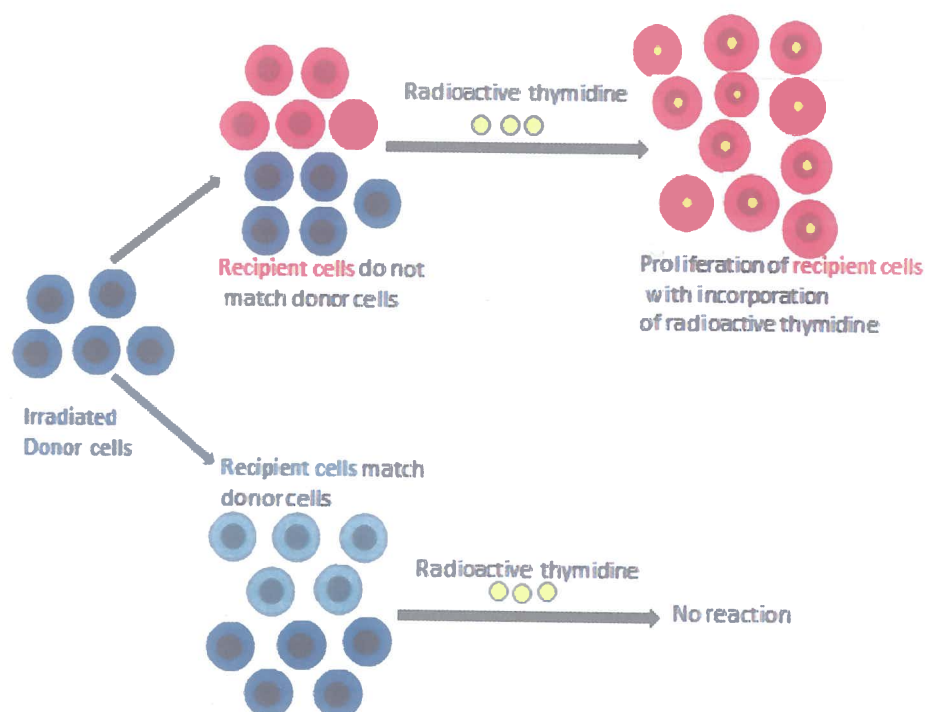
# Prevention of Rejection



**b) Compatibility testing by the mixed lymphocyte reaction (MLR): (Fig. 51)**

This test is used to test the responsiveness of the recipient lymphocytes to antigens expressed on donor cells:

- Donor and recipient cells are incubated together.
- Recognition of foreign HLA antigens on donor cells leads to proliferation of the recipient's T cells.  
(Proliferation of donor cells in response to recipient cells is prevented by previous irradiation of donor cells).
- The amount of proliferation can be measured by the extent of uptake of radioactive thymidine: the greater the extent of proliferation, the higher the disparity between donor and recipient. Low responses are associated with excellent transplant survival.
- In case of bone marrow transplantation, it is important to assess response of donor lymphocytes to recipient antigens, in order to avoid GVHD.  
(In this case, it is the recipient cells that are irradiated and it is the proliferation of donor cells that is measured.)



**Fig. (51): Mixed lymphocyte reaction**

**II. Immunosuppressive Therapy:**

This is required to prevent and/or treat graft rejection and GVHD. It may be used as a maintenance therapy or in treating episodes of rejection. These drugs carry the risk of infection as a consequence of immunosuppression.

- *Anti-inflammatory agents:*  
e.g. corticosteroids
- *Inhibitors of cytokine production:*

Cyclosporine and tacrolimus inhibit T cell cytokine production (mainly IL-2 and IFN- $\gamma$ ).

- *Anti-proliferative drugs:*  
Azathioprine and methotrexate inhibit DNA production, thus, preventing lymphocyte proliferation.
- *Monoclonal antibodies:*  
For example, monoclonal anti-CD3 antibodies block functions of T cells.

### III. Antigen-specific induced Tolerance:

Induction of tolerance to graft antigens without affecting protective immune responses is still under trial.

#### MCQs:

- 1- **The MHC is characterized by all the following EXCEPT:**
  - a- It codes for the human leukocyte antigens (HLA).
  - b- It determines the compatibility of donor and recipient tissues.
  - c- It may increase the susceptibility of individuals to autoimmune diseases.
  - d- It is co-dominantly expressed from maternal and paternal chromosomes.
  - e- Its products are not involved in allograft rejection.
- 2- **The following is true regarding acute rejection EXCEPT:**
  - a- It is the most common type of rejection.
  - b- It is antibody-mediated.
  - c- It takes days or weeks to develop.
  - d- It is explained by HLA incompatibility.
  - e- It is one of the types of allograft rejection.
- 3- **Bone marrow transplantation in immunocompromised patients presents which major problem?**
  - a- Possibility of graft-versus-host disease
  - b- High risk of T cell leukaemia
  - c- Inability to use a live donor
  - d- Delayed hypersensitivity
  - e- None of the above
- 4- **In the lymphocytotoxicity test, the following are used EXCEPT:**
  - a- Antibodies against different HLAs
  - b- Lymphocyte suspension under test
  - c- Vital dye
  - d- Radioactive thymidine
  - e- Complement
- 5- **An approach under trial for the prevention of graft rejection is:**
  - a- Antigen-specific induced tolerance
  - b- Antigen-specific immuno-stimulation
  - c- Immunosuppression
  - d- Better selection of the donors
  - e- Adjuvants



## TOLERANCE AND AUTOIMMUNITY

### ILOs:

By the end of this chapter the student should be able to:

- Define immunologic tolerance and autotolerance
- Represent the central and the peripheral mechanisms of T and B cell tolerance
- Define acquired (induced) tolerance
- Recognize the factors influencing induction of tolerance
- Recall the etiology and different mechanisms of autoimmunity
- Explain the role of genetic predisposition in the development of autoimmune diseases
- Identify the clinical spectrum of autoimmune diseases
- Explain the mechanisms of tissue damage in autoimmune diseases
- Summarize the laboratory tests for diagnosis of autoimmune diseases
- Identify the therapeutic measures of autoimmune diseases

## TOLERANCE

Tolerance is the absence of specific immune response against some antigens in an otherwise **fully immunocompetent person**. It includes autotolerance and acquired (induced) tolerance.

### I. Autotolerance

Autotolerance is a tolerance to self-antigens. Failure of autotolerance may result in autoimmune disease.

#### Mechanism of Autotolerance

##### 1. Central tolerance

It is proposed that during development in the primary lymphoid organs, B and T lymphocytes go through a phase in which contact with antigen leads to their death or permanent inactivation. Such antigens are most likely to be self-antigens. The elimination of immature self-reactive lymphocytes during their maturation is called **negative selection (clonal deletion)** (Fig. 52).

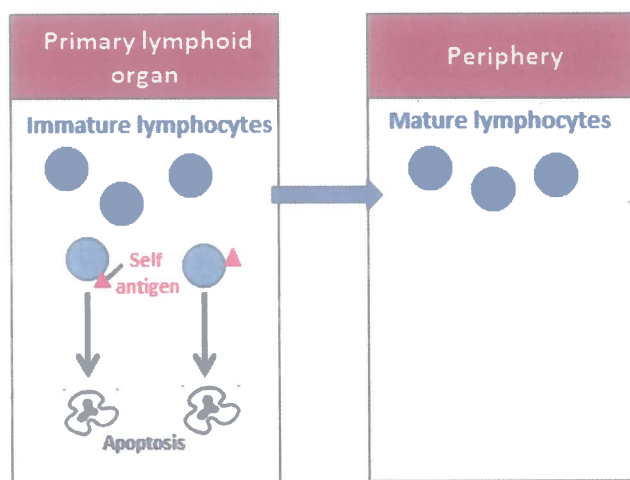


Fig. (52): Negative selection (clonal deletion)

## 2. Peripheral tolerance

- Sometimes elimination of self-reactive cells in the primary lymphoid organs is not complete. This is probably because not all self-antigens are expressed in the primary lymphoid organs; therefore, the developing lymphocytes do not encounter them.
- Self-reactive B cells may reach maturity and migrate to the secondary lymphoid organs. However, they are controlled by lack of T cell help (Th cells specific to this particular antigen have been deleted).
- As with B cells, not all self-reactive T cells are deleted in the thymus. Such T cells are normally rendered unable to respond by various mechanisms which prevent their stimulation, such as Tregs and Ts cells.

## II. Acquired (Induced) Tolerance

This is the induction of tolerance to an antigen at any time during life. It can be used as an approach for the therapy of autoimmune diseases, allergic conditions and graft rejection. The antigen is given in a certain form and under certain circumstances which favour the development of tolerance. Such an antigen is called a **tolerogen**.

### Factors influencing the induction of tolerance

1. High doses of antigen usually tolerize B cells, while minute doses given repeatedly tolerize T cells. A moderate dose of the same antigen might be immunogenic.
2. Protein antigens are more tolerogenic when in a soluble form than in aggregated or particulate form.
3. The tolerogen must persist or be repeatedly administered for acquired tolerance to be maintained.
4. Giving the antigen together with an immunosuppressant, such as cyclosporine, favours the development of tolerance.
5. Induction of tolerance is easier in the prenatal or neonatal period because of immaturity of the immune system.

**N.B.:** T cell tolerance is more easily induced and lasts longer than B cell tolerance.

## AUTOIMMUNITY

It is an adaptive immune response to self-antigens. Normally, this is prevented by autotolerance. Breakdown in autotolerance leads to production of autoantibodies and/or self-reactive T cells which may cause **autoimmune diseases**.

### Aetiology of Autoimmune Diseases

Undoubtedly, autoimmune diseases have a multifactorial aetiology. There are a number of ways in which the autotolerance mechanisms could be overcome:

1. Exposure of the immune system to antigens that are normally sequestered within organs, e.g. eye lens and sperms. Lymphocytes specific to such antigens were not exposed to them during development and, therefore, were not deleted. For example, testicular trauma or infection may release sperm antigens which trigger the immune system. This results in an autoimmune reaction that may end in damage of the testicular tissue and sterility (autoimmune orchitis).
2. Structural modification or alteration of tissue proteins by drugs, chemicals or viruses, so that such antigens are no longer recognized as self. For example,  $\alpha$ -methyl dopa is thought to modify proteins on the surface of red cells, leading to autoimmune haemolytic anaemia.
3. Cross reactivity: Some strains of *Streptococcus pyogenes* share antigenic determinants with the heart tissue. Antibodies produced against such strains may react with heart tissue leading to rheumatic fever (Fig. 53)

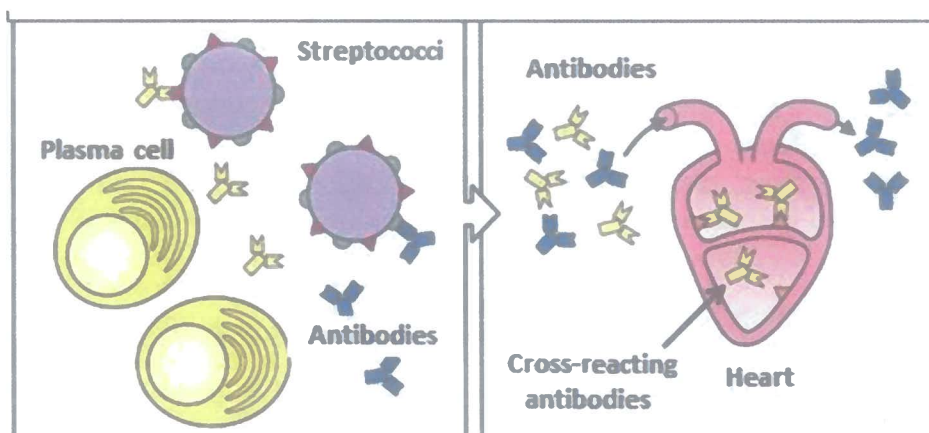


Fig. (53): Rheumatic fever caused by cross-reacting antibodies

4. Breakdown in the immune network which may occur as a result of:
  - Interference with the mechanisms which normally suppress surviving self-reactive T cells.
  - Polyclonal activation of lymphocytes: Certain agents (e.g. viruses or bacteria) are capable of non-specifically stimulating many clones of lymphocytes, including self-reactive clones.
  - Over production of IL-2 by Th1 cells



## Genetic predisposition to autoimmune diseases

Genetic factors appear to play a role in the development of autoimmune diseases, and autoimmune diseases are found to run in families. This is thought to be related to specific HLA (MHC) antigens which are important in presentation of antigens to T cells. There are strong associations between several autoimmune diseases and particular HLA specificities.

Examples include:

- Systemic lupus erythematosus (SLE) and DR3
- Rheumatoid arthritis and DR4
- Ankylosing spondylitis and B27

## Spectrum of Autoimmune Diseases

There is a wide spectrum of autoimmune disorders:

- At one extreme, there are the organ-specific diseases where the immune response is directed against components specific to the organ involved. An example of this is autoimmune thyroid diseases: Grave's disease, Hashimoto's disease and myxoedema.
- At the other end of the autoimmune spectrum are the non-organ-specific diseases where the lesion is not confined to one organ. Examples of such diseases are SLE and rheumatoid arthritis.
- In between are diseases in which antibodies formed are non-organ specific but the lesion tends to localize to a single organ e.g. antimitochondrial antibody in primary biliary cirrhosis.

A number of patients with autoimmune diseases tend to suffer from more than one condition.

## Mechanisms of Pathogenesis in Autoimmune Disorders

1. Cytotoxic reactions (type II) as in:

- autoimmune haemolytic anaemia, where antibodies are formed against RBCs, resulting in haemolysis
- thyrotoxicosis (Graves' disease), where antibodies are formed against TSH receptors, leading to their stimulation and overproduction of thyroxine (Fig. 54)

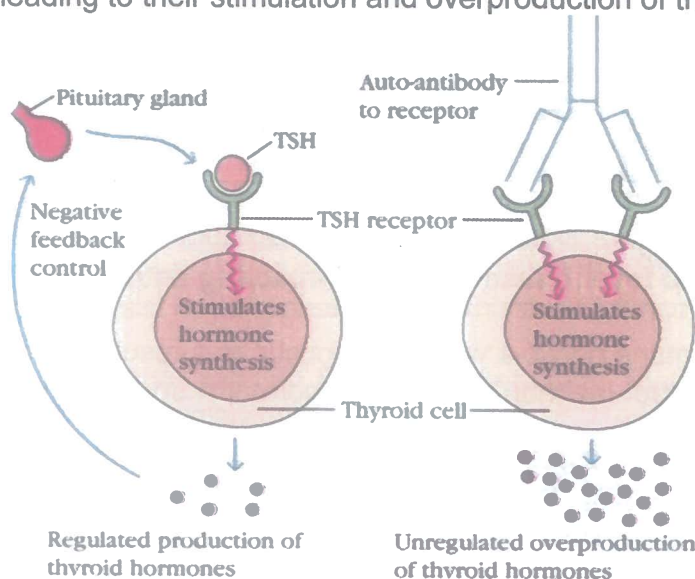


Fig. (54): Pathogenesis of Graves' disease

- myasthenia gravis, where antibodies are formed against acetyl choline receptors in muscles, resulting in blocking of neuromuscular transmission (Fig. 55)

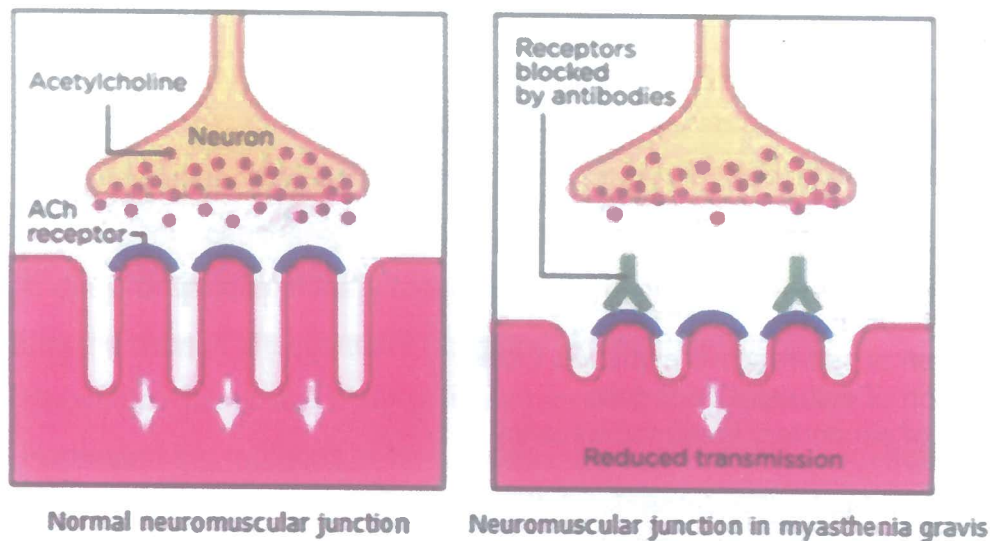


Fig. (55): Pathogenesis of myasthenia gravis

2. Immune complex deposition (type III) as in SLE and rheumatoid arthritis
3. Cell-mediated reactions (type IV) as in cases of ulcerative colitis

### Laboratory diagnosis of Autoimmune Diseases

1. Elevated level of serum immunoglobulins
2. Detection of autoantibodies in the serum

Examples include:

- rheumatoid factor (IgM directed against self-IgG)
  - antinuclear antibodies (ANA)
  - anti-DNA antibodies
  - anti-mitochondrial antibodies
  - anti-smooth muscle antibodies
  - anti-thyroid antibodies
3. Detection of immune complexes in the serum and in tissue biopsy
  4. Decreased level of serum complement, due to uptake in immune complexes

### Management of Autoimmune Diseases

1. Anti-inflammatory drugs, e.g. corticosteroids
2. Immunosuppressive drugs, e.g. methotrexate
3. Plasmapheresis: clearing the plasma from immune complexes and antibodies
4. Interference with the cytokine network is under trial.

**MCQs:**

- 1- **Elimination of immature self-reacting lymphocytes in the primary lymphoid organs is called:**
  - a- Acquired tolerance
  - b- Central tolerance
  - c- Peripheral tolerance
  - d- Clonal selection
  - e- Positive selection
- 2- **The mechanisms of autotolerance involve:**
  - a- Immunosurveillance
  - b- Clonal deletion
  - c- Activation of self-reactive cytotoxic T cells
  - d- Activation of self-reactive B cells
  - e- A state of secondary immunodeficiency
- 3- **Negative clonal selection (clonal deletion) is:**
  - a. One of the mechanisms of peripheral tolerance
  - b. More probable to occur later in life
  - c. The elimination of immature self-reactive lymphocytes during their maturation
  - d. The elimination of immature non-self-reactive lymphocytes during their maturation
  - e. The elimination of mature self-reactive lymphocytes
- 4- **Which one of the following diseases is a non-organ specific autoimmune disease?**
  - a- Grave's disease
  - b- Myxoedema
  - c- Hashimoto's thyroiditis
  - d- Systemic lupus erythromatosis
  - e- Autoimmune haemolytic anaemia
- 5- **Rheumatoid factor is:**
  - a- DNA-anti-DNA immune complex
  - b- Autoantibody to IgM
  - c- Autoantibody to complement components
  - d- Autoantibody to IgG
  - e- Any factor predisposing to rheumatoid arthritis
- 6- **The laboratory diagnosis of autoimmune diseases includes all the following EXCEPT:**
  - a- Decreased level of serum complement
  - b- Decreased level of serum immunoglobulins
  - c- Detection of immune complexes in serum
  - d- Detection of immune complexes in tissues
  - e- Detection of serum auto-antibodies



## IMMUNODEFICIENCY DISEASES

### ILOs:

By the end of this chapter the student should be able to:

- Classify immunodeficiency diseases
- Explain the causes, genetic mechanisms, clinical and immunological findings of various primary (congenital) immunodeficiency diseases
- List the causes of secondary (acquired) immunodeficiency
- Recognize the clinical findings in immunodeficiency diseases
- Represent the methods of investigation of a case of immunodeficiency
- State the general and specific measures of management of immunodeficient patients

In view of the complex nature of the immune response, it is not surprising that a wide array of immunodeficiency diseases exist. The individual may be born with an immune defect (**primary immunodeficiency**) (Table 13).

More commonly, he may acquire a transient or permanent immunologic impairment later in life (**secondary immunodeficiency**) (Fig. 56).

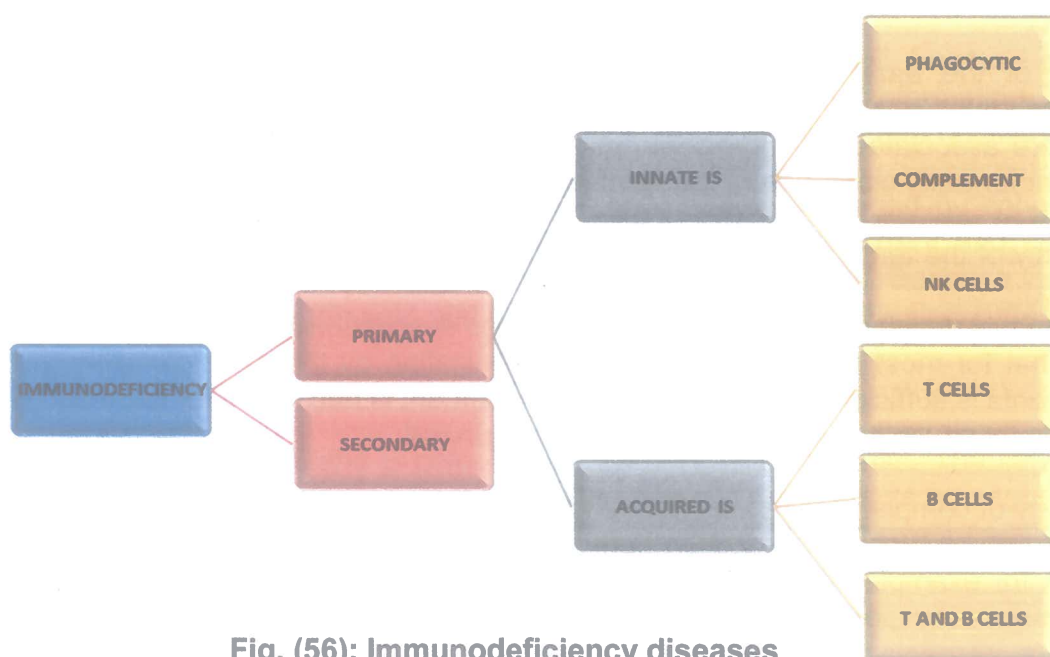


Fig. (56): Immunodeficiency diseases

## A- Primary Immunodeficiency

### I. Defects of the Innate Immune System

#### 1. Phagocytic cell defects:

These may involve any of the stages of phagocytic function.

##### a- Defects of migration (mobility):

An example of this condition is **leucocyte adhesion deficiency**, which involves a defect in **adhesion molecules**. This leads to failure of neutrophils and monocytes to migrate from the blood stream to sites of infection, despite their presence in large numbers in the blood. Patients present with **bacterial** infections of the skin, mouth and respiratory tract, but with little pus formation (because pus is largely formed of neutrophils).

**b- Defects of engulfment:** These are usually associated with defects of mobility and are rarely found alone.

**c- Defects of intracellular killing: Chronic granulomatous disease (CGD)** is an important example. In this condition, phagocytes cannot produce reactive oxygen radicals, leading to decreased ability to kill intracellular as well as ingested extracellular bacteria. It is usually inherited as an X-linked disease. Affected male children present with chronic **bacterial** and **fungal** infections which may be associated with lesions resembling granulomas.

#### 2. Complement deficiencies

a- Defects of the early components of the classical pathway of complement activation:

These components are important for the elimination of immune complexes. Thus, their deficiency leads to accumulation of immune complexes, and local tissue damage.

b- Defects of the early components of the alternative pathway of complement activation:

These are associated with **pyogenic** infections, since the alternative pathway is important for early clearance of these infections.

c- Deficiency of the terminal components of complement (C5-C9) (Membrane Attack Complex):

Effects of this deficiency are confined to increased susceptibility to **Neisseria**. This shows that for most extracellular bacteria opsonization by the early complement components is sufficient, whereas lysis by complement is necessary for combating **Neisseria**, because it is capable of intracellular survival.

d- Deficiency of complement regulatory proteins:

Absence of complement regulation can cause many problems. Deficiency of C1 inhibitor, for example, leads to uncontrolled activation of the classical pathway of complement activation, starting with C1, followed by C2 activation and generation of C2a (a vasoactive amine). This may cause increased vascular permeability, leading to painful swelling at any site. Patients suffer from bouts of facial swelling

and abdominal pain. If the larynx is affected it may lead to suffocation and death. The condition is known as **hereditary angioedema**.

### 3. Deficiency of NK cells

This is usually present in conjunction with other defects and rarely alone. Since NK cells are important in anti-viral and anti-tumour immunity, patients with NK cell defects suffer from certain **viral** diseases and **malignancies**.

## II. Defects of the Acquired Immune System

### 1. B cell immunodeficiency (antibody immunodeficiency)

The primary antibody immunodeficiency disorders range from complete absence of all classes of antibodies to selective deficiency of a single class or subclass of antibody.

a- **X-linked agammaglobulinaemia**: is a severe form of antibody immunodeficiency with marked deficiency or complete absence of all classes of antibodies. Infants with this condition usually become symptomatic following natural loss of maternal antibodies at the age of 5-6 months. They suffer from severe chronic **bacterial** infections such as otitis, bronchitis and pneumonia. Extracellular bacteria are responsible for most of these infections. Patients have a normal T cell response to viral infections, but susceptibility to some viruses may be increased due to the absence of neutralizing antibodies.

b- **Transient Hypogammaglobulinaemia of Infancy (THI)**: is another example. All infants develop physiological hypogammaglobulinaemia at 5 - 6 months of age when maternal IgG decreases and the infants start synthesizing their own IgG. Sometimes, the infant may fail to start IgG synthesis at this time, resulting in a prolonged period of THI. Such infants have recurrent infections and poor response to the vaccines taken routinely at that age.

### 2. T cell immunodeficiency

Defective T cell immunity usually affects humoral immunity as well, because of the collaboration between T cells and B cells in the process of antibody formation. Thus, production of antibodies against T-dependent antigens in particular can be affected in cases of T cell immunodeficiency.

**Di George syndrome** is a severe form of T cell deficiency caused by congenital aplasia or hypoplasia of the thymus. The patients develop recurrent and chronic infections with **intracellular pathogens** (mainly viruses and fungi). Because there is usually aplasia of the parathyroid gland as well, such patients may also suffer from tetany.

### 3. Combined T and B cell deficiency

This involves defective T and B cells. It may be complete or partial. The complete form is called **severe combined immunodeficiency (SCID)**. Patients present during the first few months with failure to thrive, continuous diarrhoea, as well as infection with **all pathogens (bacterial, viral, fungal and protozoal infections)**.



**Table (13):** Summary of important primary immunodeficiency conditions

Deficient component and name of disease	Specific deficiency	Clinical features
<b>1) Phagocytes:</b> -Leucocyte adhesion deficiency ( <b>LAD</b> )  -Chronic granulomatous disease ( <b>CGD</b> )	Defect in migration due to defect in adhesion molecules  Defects of intracellular killing due to failure in production of reactive O <sub>2</sub> radicals	Bacterial infections of skin, mouth and respiratory tract, but with <b>no pus</b> formation  Chronic bacterial and fungal infections
<b>2) Complement:</b> -Early components of the classical pathway  -Early components of the alternative pathway  -(C5-C9) (Membrane Attack Complex)  -Regulatory proteins (Hereditary angioedema)	Defect in clearance of immune complexes  Defect in clearance of pyogenic infections  Defect in lysis by complement  Defect in C1 inhibitor	Accumulation of immune complexes, and local tissue damage.  Pyogenic infections  <i>Neisseria</i> infections  Bouts of facial swelling, abdominal pain and possibly suffocation
<b>3) B cells:</b> -X-linked agammaglobulinaemia  -Transient hypogammaglobulinaemia of Infancy ( <b>THI</b> )	Complete absence of all classes of antibodies  Delayed production of IgG by infant	Severe chronic bacterial infections  Recurrent infections and poor response to vaccines
<b>4) T cell:</b> -Di George syndrome	Severe T cell deficiency due to thymic aplasia	-Recurrent and chronic infections with intracellular pathogens (mainly viruses and fungi) -Tetany
<b>5) Combined B &amp; T cells:</b> - Severe combined immunodeficiency ( <b>SCID</b> )	Complete deficiency of both B and T cell function	Bacterial, viral, fungal and protozoal infections

## B- Secondary Immunodeficiency

Several disorders are associated with secondary immunodeficiency:

1. **Malnutrition** is the leading cause of immunodeficiency in the world as a whole, and is of particular importance in poor and under-developed countries.
2. **Human immunodeficiency virus (HIV)** causes **acquired immune deficiency syndrome (AIDS)**. The virus infects CD4 Th cells, as well as macrophages and dendritic cells. Because cell mediated immunity is affected, patients suffer from infections with intracellular pathogens. Humoral response to T-dependent antigens may also be affected.
3. Other viral infections may also cause transient immunodeficiency, e.g. measles.
4. Severe bacterial infections, e.g. tuberculosis
5. Parasitic infestations, e.g. shistosomiasis
6. Malignancies, especially those affecting the immune system such as Hodgkin's disease and leukaemias
7. Chronic debilitating diseases, e.g. diabetes and renal failure
8. Others, e.g. treatment with x-ray, cytotoxic drugs, steroids and immunosuppressive drugs, and burns with severe loss of body fluids

### When to Suspect Immunodeficiency ?

Immunodeficiency should be suspected in the following cases:

- Increased frequency of infections
- Failure to clear infections rapidly despite adequate therapy
- Dissemination of local infections to distant sites
- Occurrence of opportunistic infections
- Failure to thrive in infants and children
- Development of certain kinds of tumours

It should be remembered that the type of infection provides an important clue to the type of immunodeficiency disease.

### Investigations in a Suspected Case of Immunodeficiency

#### Initial investigations

- Full blood picture
- Measurement of serum IgG, IgM, IgA and C3 by radial immunodiffusion

#### Detailed Investigations

If initial tests are normal and high level of suspicion still exists, or if the initial tests point to a certain direction, then more specialized tests can be done:

##### T lymphocytes

- Quantitation of cells by fluorochrome-labelled monoclonal antibodies against cell markers, e.g. with antibodies against CD3 (to count total T cells) and against CD4 and CD8 (to count T cell sub-sets):

Cells can be counted using the fluorescent microscope, or by flow cytometry.

*Flow cytometry is a modification of immunofluorescence. Labelled cells are passed through an apparatus called the **flow cytometer** which can define cells according to morphology and according to the fluorescent labels attached to them. All data is plotted and displayed in a graph, enabling different populations of cells (e.g. CD8+ or CD4+) to be counted.*

- Measuring the ability of T cells to proliferate in response to mitogens (Mitogens are substances that can non-specifically stimulate proliferation of lymphocytes. Some stimulate T cells only, some B cells only, while others stimulate both.)
- Delayed type hypersensitivity tests to test the functional activity of T cells: Tests are done using extracts from organisms to which all people are frequently exposed, e.g. candidin test.
- Assessment of cytokine production

### **B lymphocytes**

- Quantitation of cells using fluorochrome-labelled monoclonal antibodies against B cell markers:  
Fluorescent microscope or flow cytometer can be used, as for T cells.
- Determination of serum level of immunoglobulin classes as well as IgA in saliva
- Immunization with commonly used vaccines such as tetanus toxoid followed by measuring the antibody response

### **Complement**

More detailed complement assays including total haemolytic assay of complement in serum:

This involves measurement of the ability of test serum to cause lysis of RBCs coated with antibody, indicating the amount of functioning complement in the serum.

### **Phagocyte functions**

Different tests can be done to assess phagocyte mobility, metabolic functions and ability to kill organisms.

## **Management of the Immunodeficient Patient**

### **General measures**

- Minimizing infections by avoidance
- Administration of suitable immunizations:  
It should be noted that patients suspected of suffering from immunodeficiency should never be given vaccines containing living organisms, since this may be fatal.
- Prompt treatment of infections with antibiotics

### **Specific Measures according to defect**

- Transplantation of foetal thymus or bone marrow in SCID and other severe deficiencies
- Immunoglobulin therapy for cases of antibody deficiencies
- Treatment with cytokines such as GM-CSF, IFN- $\gamma$  and IL-2

*Immunodeficiency disorders can be considered as "experiments of nature" which have helped scientists to understand the role of each component of the immune system against various infectious agents.*



**MCQs:**

- 1- **Leucocyte adhesion deficiency is an example of a defect in:**
  - a- Migration
  - b- Engulfment
  - c- Intracellular killing
  - d- The number of leucocytes
  - e- Terminal complement components
- 2- **C1 inhibitor deficiency results in:**
  - a- Increased susceptibility to pyogenic infections
  - b- Increased susceptibility to infection with *N. meningitidis*
  - c- Hereditary angioedema
  - d- Infections characterized by little pus formation
  - e- Chronic infections with granuloma formation
- 3- **Deficiency of NK cells may manifest by:**
  - a- Increased incidence of pyogenic infections
  - b- Increased incidence of hypersensitivity reactions
  - c- Increased rejection of transplanted organs
  - d- Increased incidence of malignancies
  - e- Decreased incidence of viral infections
- 4- **X-linked agammaglobulinaemia is characterized by deficiency of:**
  - a- IgG
  - b- IgM
  - c- IgA
  - d- All of the above
  - e- All classes of complement
- 5- **An X-ray film revealed absence of the thymus in an infant. The most likely diagnosis of this case is:**
  - a- DiGeorge syndrome
  - b- Chronic granulomatous disease
  - c- Severe combined immunodeficiency
  - d- Leukocyte adhesion deficiency
  - e- X-linked agammaglobulinaemia
- 6- **The functional capability of T cells can be assayed by:**
  - a- Mixed lymphocyte reaction
  - b- Fluorescent antibody assay with CD8 antiserum
  - c- Fluorescent antibody assay with CD3 antiserum
  - d- *In vitro* response to mitogens
  - e- *In vivo* response to toxoids

## Answers

<b>Chapter 1:</b>	1 d	2 c	3 a	4 e	5 c	6 c	
<b>Chapter 2:</b>	1 d	2 d	3 b	4 b	5 b		
<b>Chapter 3:</b>	1 c	2 d					
<b>Chapter 4:</b>	1 c	2 c	3 d	4 d	5 e	6 c	7 d
<b>Chapter 5:</b>	1 d	2 e	3 d	4 c	5 c	6 e	7 d
<b>Chapter 6:</b>	1 e	2 b	3 c	4 e	5 d	6 a	7 b
<b>Chapter 7:</b>	1 d	2 c	3 e	4 a			
<b>Chapter 8:</b>	1 c	2 c	3 b	4 e	5 b		
<b>Chapter 9:</b>	1 e	2 c	3 a	4 c	5 c		
<b>Chapter 10:</b>	1 c	2 e	3 a	4 a			
<b>Chapter 11:</b>	1 e	2 c	3 b	4 a	5 c	6 b	
<b>Chapter 12:</b>	1 e	2 b	3 a	4 d	5 a		
<b>Chapter 13:</b>	1 b	2 b	3 c	4 d	5 d	6 b	
<b>Chapter 14:</b>	1 a	2 c	3 d	4 d	5 a	6 d	



# ESSENTIAL MEDICAL MICROBIOLOGY and IMMUNOLOGY

Volume III





**ESSENTIAL  
MEDICAL MICROBIOLOGY  
AND IMMUNOLOGY**

**VOLUME III**  
**Systematic Bacteriology**

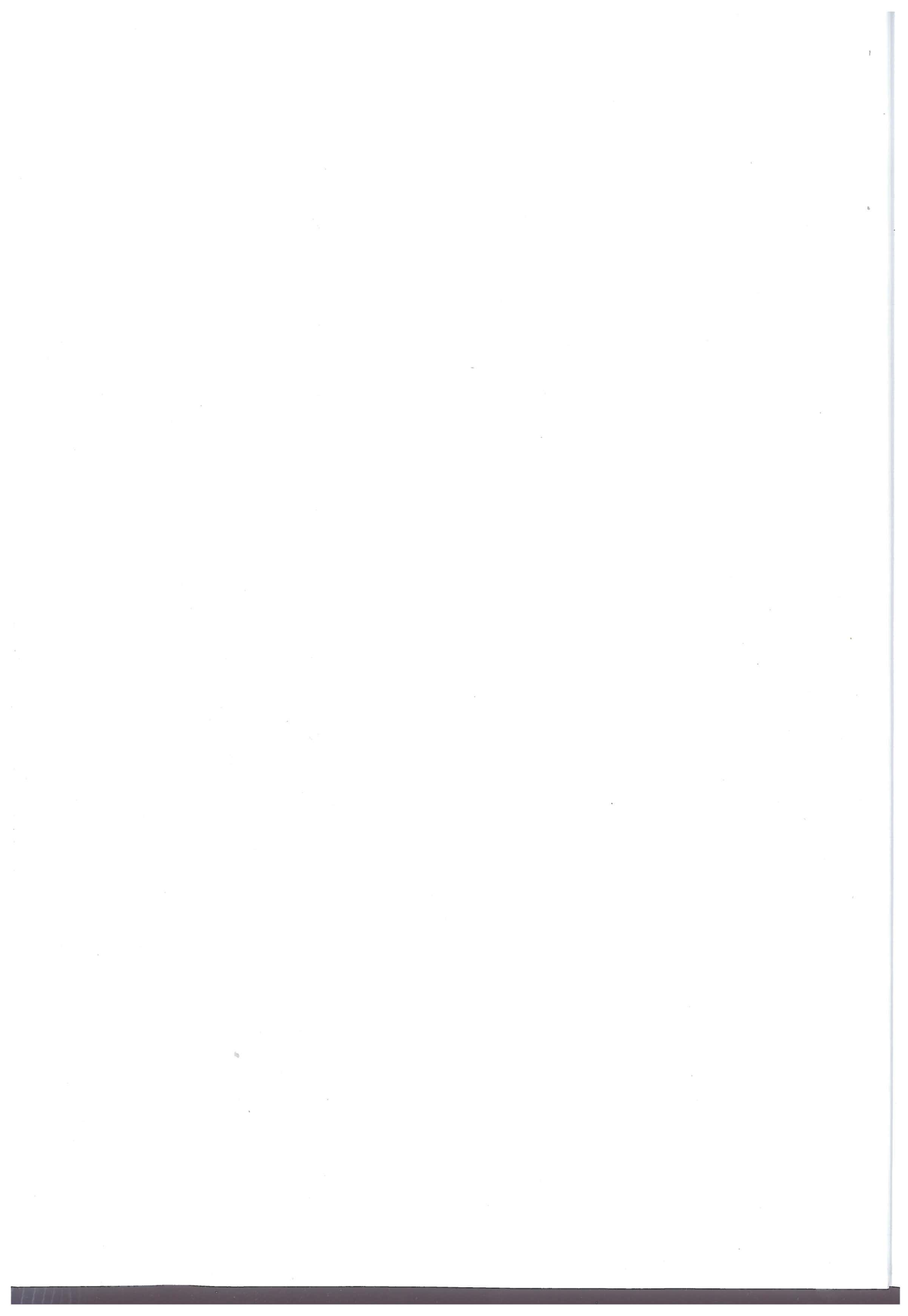
**Eighth Edition**

**By**

***Staff Members of  
Medical Microbiology and Immunology Department***

**Faculty of Medicine-Cairo University**

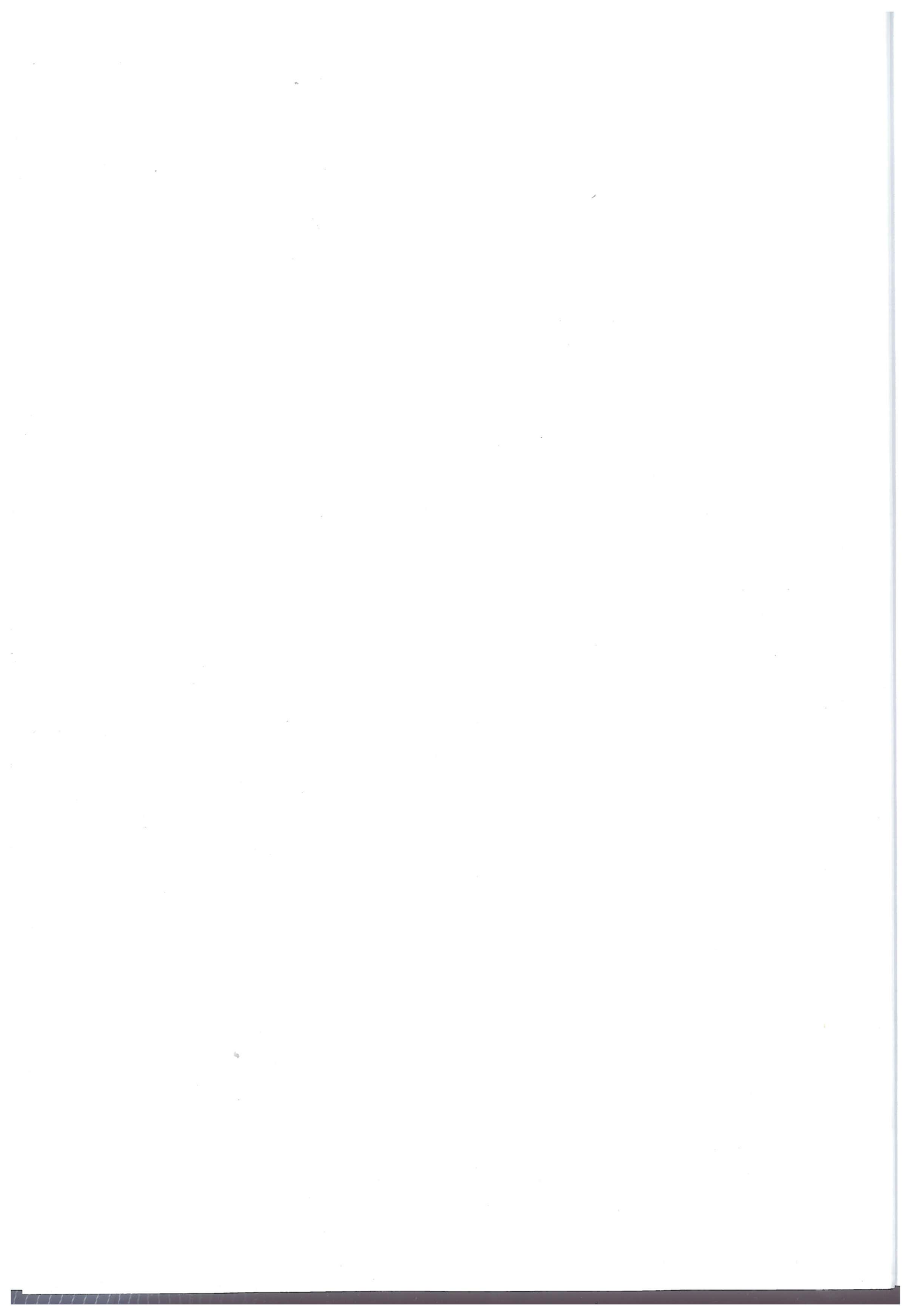
**2018-2019**





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# MICROBIAL TAXONOMY

**ILOs:**

By the end of this chapter the student should be able to:

- Define the terms taxonomy, classification, nomenclature and identification
- Recognize taxonomic groups
- List criteria for classifying bacteria

Taxonomy (taxon = group) is the science of biological classification. It consists of three parts: **classification**, **nomenclature**, and **identification**:

- **Classification** is the arrangement of organisms into groups.
- **Nomenclature** refers to the assignment of names to taxonomic groups.
- **Identification** refers to the determination of the particular taxon to which a particular isolate belongs.



## Taxonomic Groups

Organisms (except viruses) are placed in taxonomic groups which include Domain, Kingdom, Phylum, Classes, Orders, Families, Genera, Species and strains in a descending order. Viruses are classified differently, so that the family is the highest taxonomic group.

The basic taxonomic group is the species. Species are defined on the basis of phenotypic and genotypic differences among bacteria.

- A **bacterial species** is a collection of strains that share many stable properties and differ significantly from other groups of strains.
- A **strain** is an individual member within the species.
- A **genus** is a well-defined group of one or more species that is clearly separate from other genera.

The **binomial system** is used for nomenclature of organisms (except viruses). It employs the genus and species names. The genus name is always capitalized (e.g. *Escherichia*) while the species name is never capitalized (e.g. *coli*); both terms are written in italic form (e.g. *Escherichia coli*). After first usage in a manuscript, the first name will often be abbreviated to the first letter (e.g. *E. coli*).

## Criteria for Classifying Bacteria

Classification is based on several parameters:

1. **Genetic homology:** is a similarity between the DNA or the RNA of organisms. It may be determined by base composition, nucleotide sequence or DNA hybridization rates.

Accordingly, two organisms are considered to belong to the same species if they have:

- DNA homology of  $\geq 70\%$  , or
- 16S rRNA sequences are  $>97\%$  identical.

2. **Phenotypic properties:** Properties such as biochemical reactions, chemical composition, cellular structures and immunological features are used in defining a bacterial species.

**MCQs:**

**1- A collection of bacterial strains that share many stable properties and differ significantly from other groups of strains is termed:**

- a- A phylum
- b- A family
- c- A species
- d- An order
- e- A genus

**2- Two organisms are considered to belong to the same species if they have:**

- a- 10% DNA homology
- b- 50% DNA homology
- c- 70% or more DNA homology
- d- At least 90% DNA homology
- e- No DNA homology

## STAPHYLOCOCCUS

### ILOs:

By the end of this chapter the student should be able to:

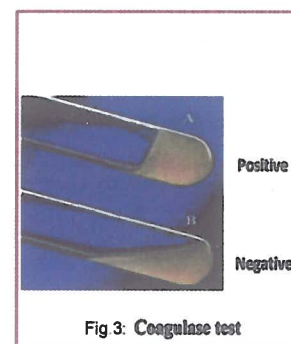
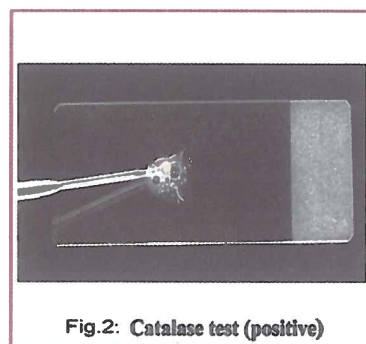
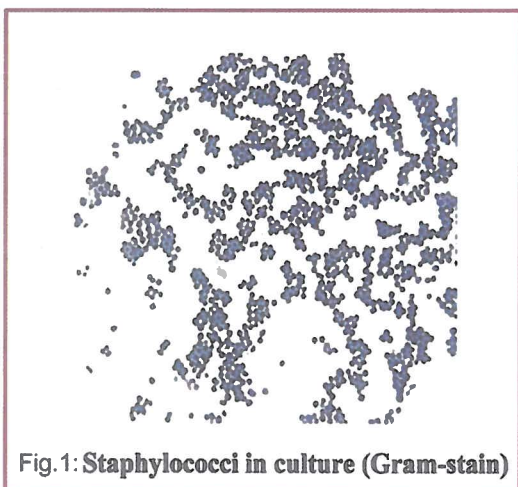
- Describe the morphology and classify species of *Staphylococcus*
- Describe culture characteristics & growth requirements of *S. aureus* and coagulase negative staphylococci
- Describe virulence factors produced by *S. aureus* and coagulase negative staphylococci
- List & differentiate staphylococcal diseases
- Outline laboratory diagnosis of infections caused by *S. aureus* and coagulase negative staphylococci
- Outline treatment for staphylococcal diseases and discuss methicillin resistant *S. aureus* including its clinical problem

### Characters of the genus *Staphylococcus*

1. Gram-positive spherical cocci arranged in grape-like clusters. (Fig.1)
2. Catalase positive. (Fig.2)
3. Opaque, pigmented colonies are usually produced on agar.

The ability to produce **staphylocoagulase** divides the genus into two groups: (Fig.3)

- Coagulase-positive staphylococci: *S. aureus* has the greatest pathogenic potential and is the most medically important species.
- Coagulase-negative staphylococci: e.g., *S. epidermidis* and *S. saprophyticus* which are far less pathogenic.





## *Staphylococcus aureus*

### Morphology

*S. aureus* strains, like other staphylococci, are Gram-positive spherical cocci (about 1  $\mu\text{m}$  in diameter) occurring in irregular grape-like clusters.

### Cultural characters

- *S. aureus* is a facultative anaerobe.
- It usually produces golden yellow endopigment. (Fig.4)
- It is usually grown on:
  - Nutrient agar.
  - Blood agar, producing complete ( $\beta$ -) haemolysis due to production of haemolysins. (Fig.5)
  - Mannitol salt agar (selective indicator medium) producing yellow colonies due to mannitol fermentation. This medium facilitates isolation of *S. aureus* (salt tolerant) from specimens contaminated by other bacteria. (Fig.6)

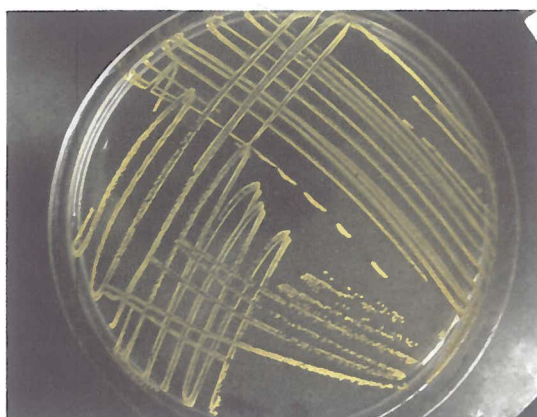


Fig.4: *S. aureus* on nutrient agar with golden yellow endopigment



Fig.5: *S. aureus* on blood agar with complete ( $\beta$ ) haemolysis

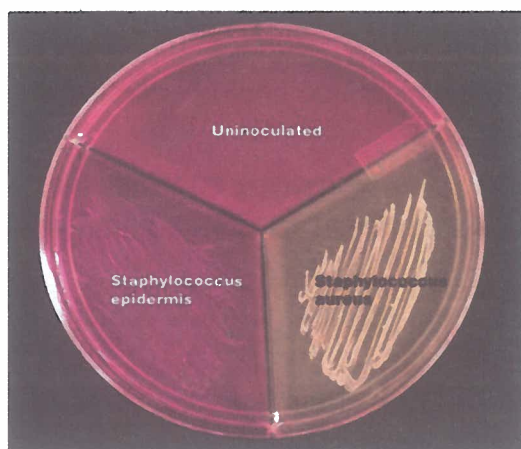


Fig.6: *S. aureus* (yellow colonies) on mannitol salt agar

## Virulence factors and pathogenesis

1. **Staphylocoagulase:** Coagulase is an extracellular protein that has the ability to convert plasma fibrinogen to fibrin. By this mechanism, a fibrin barrier is formed. This leads to:
  - Protection from phagocytic and immune defences.
  - Localization of infection e.g., furuncles.
2. The **clumping factor** (fibrinogen-binding protein): This is an important **adhesin** that leads to attachment of the organism to traumatized tissue and blood clots.
3. **Invasins:** Leucocidin, staphylokinase and hyaluronidase promote bacterial spread in tissues.
4. **Protein A:** It is present on surface of *S. aureus*. It binds non-specifically to the Fc-portion of IgG leading to inhibition of opsonization. (Fig.7)

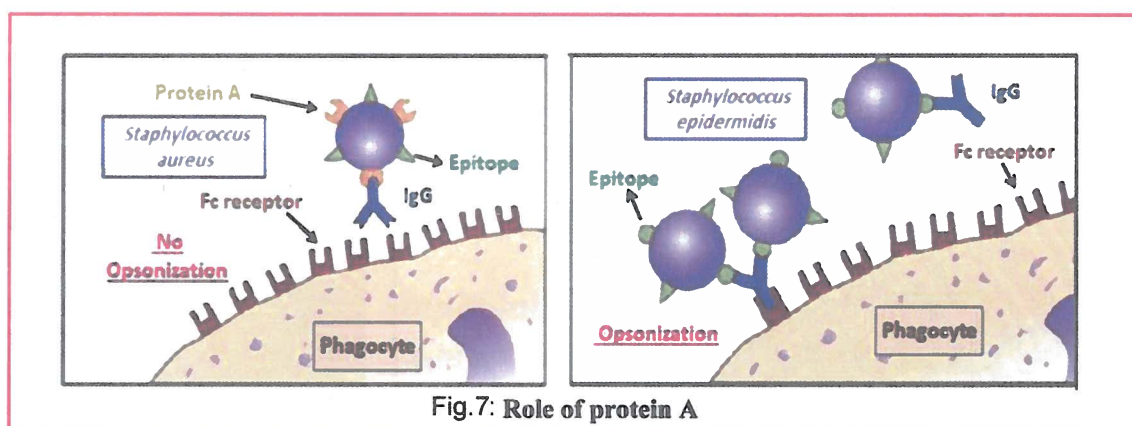


Fig.7: Role of protein A

5. **Haemolysins (e.g. alpha toxin):** These are pore-forming toxins that lyse host cell membranes. They cause haemolysis on blood agar.
6. **Exotoxins having superantigen mechanism (see vol. I):**
  - a- **Enterotoxins** responsible for staphylococcal food poisoning.
  - b- **Toxic shock syndrome toxin-1 (TSST-1).**
  - c- **Epidermolytic (exfoliatin) toxins** responsible for staphylococcal scalded skin syndrome (SSSS).

## Staphylococcus aureus Diseases

Staphylococci usually inhabit the skin (especially the perineum) and mucosa. The nose is the main habitat for *S. aureus* with a nasal carriage rate of more than 40% in adults.

The chief sources of infection are shedding human lesions, fomites contaminated from such lesions, the human respiratory tract and skin. Contact spread of infection is of significant importance in hospitals, where a large proportion of the staff and patients carry antibiotic-resistant staphylococci in the nose or on the skin. Contaminated hands of the healthcare workers account for transmission of several healthcare-associated infections.



## A- Pyogenic diseases

- I. **Localized skin infections** are by far the most common: (Fig.8,9)
  - a- Folliculitis, furuncles, carbuncles, or abscesses.
  - b- Surgical site infections.
  - c- Traumatic wound infections following skin injury and burns.
- II. **Staphylococcal pneumonia** is a frequent complication of prior viral infections (e.g. measles or influenza).
- III. **Invasive conditions** are more serious and usually occur in immuno-compromised individuals. Invasion of bloodstream (bacteraemia) and spread to numerous body sites lead to deep seated infections such as osteomyelitis, endocarditis and meningitis. A resulting septicaemia may be rapidly fatal.



Fig.8: Folliculitis (localized skin infection)



Fig.9: Staphylococcal wound infection

## B- Toxin-mediated diseases

### I. Staphylococcal food poisoning:

- It is the commonest type of bacterial food poisoning.
- Incriminated foods include protein-rich food like mayonnaise, milk and its products (e.g. ice cream), or carbohydrate-rich food e.g. pasta, cake and koskosi.
- The source of food contamination may be:
  - A carrier such as food handlers harbouring *S. aureus* on their hands or in the nose.
  - A person with pyogenic staphylococcal infection e.g., furuncle.
- The organism grows in the food and produces the toxin.
- There are at least six antigenic types of enterotoxins (A, B, C, D, E and G) produced by ~50% of *S. aureus* strains. These toxins use a superantigen mechanism.



- Staphylococcal enterotoxins do not change the characters of the food regarding its taste, colour or odour. In addition, heating may kill the organism but does not destroy the toxin since it is heat-stable (for ~30 minutes of boiling).
- Incubation period is characteristically short (1-6 hours after ingestion of foods containing **preformed toxin**).
- It manifests as violent vomiting and diarrhoea, usually without fever.
- It is usually self-limited.

## II. Toxic shock syndrome (TSS):

- TSS is due to infection or colonization by TSST1-producing *S. aureus*.
- It was first described in young menstruating females who use vaginal tampons that are left in place for extended period. However, the syndrome can also occur in any individual suffering from TSST-1 producing *S. aureus* infections anywhere in the body.
- The disease is characterized by sudden onset of high fever, diarrhoea, vomiting and red rash. Hypotension with cardiac and renal failure may occur due to the **superantigen** action of TSST-1.
- The mortality rate may reach 10-15%.

## III. Staphylococcal scalded skin syndrome (SSSS): (Fig.10)

- It occurs in neonates and children under 5 years of age.
- It follows infections caused by *S. aureus* that produces exfoliatin toxins.
- Large bullae are formed under the epidermis, which rupture leaving moist, red, scalded dermis.
- Full recovery without scar formation is the rule.



Fig.10: Staphylococcal scalded skin syndrome

## Laboratory diagnosis

**A. Specimens** may include pus, sputum, urine, CSF, blood (in cases of bacteraemia, septicaemia and endocarditis) ....etc.

**B. Direct detection in Gram-stained smears:** Gram-positive cocci are seen in clusters in association with pus cells. Microscopy cannot discriminate staphylococcal species. (Fig.11)

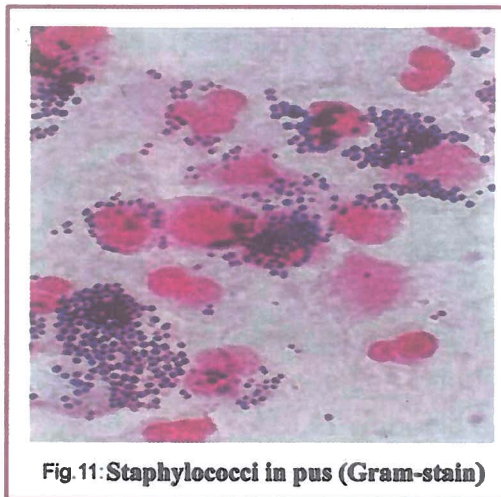


Fig.11: Staphylococci in pus (Gram-stain)

### C. Cultivation

- 1- Specimens other than the blood should be plated directly onto blood agar and mannitol salt agar and incubated at 37°C.
- 2- Blood samples should be cultivated by the blood culture technique. Subcultures are plated on blood agar and incubated as above.

### D. Identification

After 24h incubation, the growth should be examined for colony morphology, Gram stain and catalase production. *S. aureus* is identified as follows:

- 1- On blood agar: golden yellow colonies surrounded by complete haemolysis.
- 2- On mannitol salt agar: yellow colonies.
- 3- Gram-stained film: Gram positive cocci in clusters.
- 4- Catalase test: positive.
- 5- Coagulase test: positive. (Fig.12)
- 6- Test for the clumping factor: positive. (Fig.13)

**Coagulase and clumping factor** are the most important markers for identifying *S. aureus* in the laboratory.



Fig.12: Coagulase test (positive)



*S. aureus*                      *S. epidermidis*  
Fig.13: Test for clumping factor



**In case of food poisoning:**

- Specimens: food remnants, vomitus and/or faeces should be tested for the causative *S. aureus* and/or its enterotoxin.
- Isolation: on selective media such as mannitol salt agar as the specimens are usually contaminated with other bacteria.
- Detection of enterotoxin production by the isolated strains or directly in the sample is done by ELISA.

**In case of toxic shock syndrome:**

The diagnosis usually depends on:

- Clinical findings.
- Isolation of the organism from suspected sites e.g., wounds, vagina or from tampons by culture on mannitol salt agar.
- Detection of TSST-1 in the blood by ELISA.

N.B.: **Strain typing** is required in the epidemiologic studies of outbreaks of *S. aureus* diseases such as food poisoning and surgical site infections.

Strain typing can be done by colony morphology, biotype profiles, phage typing, plasmid analysis, ribotyping, chromosomal analysis and PCR. (Fig.14)

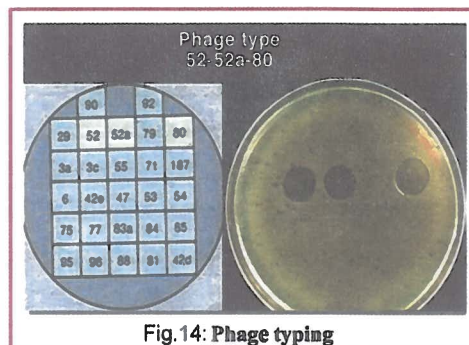


Fig.14: Phage typing

**Prevention and control**

Improved hygiene and proper infection control practices in hospitals are the most effective methods of prevention, especially hand hygiene and meticulous attention to aseptic techniques.

**Treatment and Antibiotic Susceptibilities** (Fig.15)

- **Abscesses** may require surgical drainage and antibiotic therapy to prevent dissemination.
- **Systemic infections** require vigorous antibiotic treatment.



Fig.15: Antibiotic sensitivity test (Disc diffusion method)



Therapy is seriously faced with the following antibiotic-resistance patterns of *S. aureus*:

### 1. Penicillin-resistant *S. aureus*

- Approximately 95% of *S. aureus* strains are resistant to penicillin. This type of resistance is due to  $\beta$ -lactamase (penicillinase) production.
- Resistance rate is highest among hospital strains.
- Resistant strains remain susceptible to the semi-synthetic penicillins (e.g. oxacillin and methicillin), and to cephalosporins.

### 2. Methicillin-resistant *S. aureus* (MRSA)

- It is a more serious type of resistance.
- There is a change in the **penicillin-binding protein (PBP)** which is the binding site for the antibiotic on the organism's cell wall. This type of resistance is due to the presence of *mec-A* gene on the chromosome of MRSA.
- Infections caused by MRSA strains cannot be treated with any of the beta-lactam antibiotics, except for a few MRSA-active cephalosporins. Also, MRSA isolates are often multiresistant to other antibiotics. **Vancomycin** is used as the drug of choice for treatment of MRSA infections.

### 3. Vancomycin-resistant *S. aureus*

- Unfortunately, during the last few years, some strains of MRSA displayed intermediate (VISA) or full resistance (VRSA) to vancomycin. This situation is very serious.
- The new antibiotics **linezolid** and **streptogramins** are used for treatment of infections not responding to vancomycin.

## Coagulase Negative Staphylococci

### I- *S. epidermidis*

#### Morphology and Cultural Characteristics

*S. epidermidis* is similar to *S. aureus* except in the following:

- It gives white non-haemolytic colonies on blood agar. (Fig.16)
- It is mannitol non-fermenter. (Fig.17)

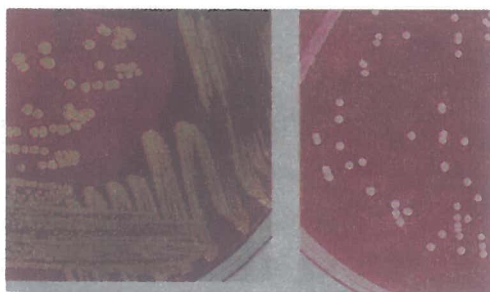


Fig.16: *S. aureus* (Golden yellow colonies) *S. epidermidis* (White colonies)

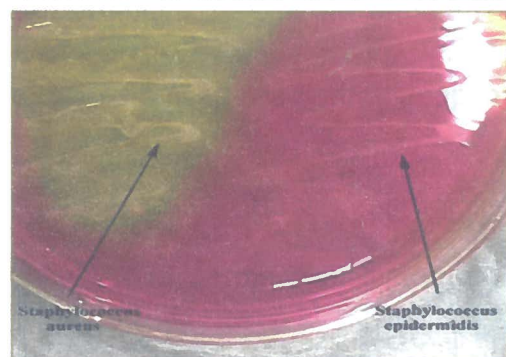


Fig.17: Mannitol salt agar

## Virulence factors

**Glycocalyx** (or slime): It is an extracellular polysaccharide which enables the organism to colonize prosthetic devices and facilitates formation of a protective **biofilm** on the device surface.

**Biofilm:** (Fig. 18)

- It is an aggregate of microorganisms in which cells adhere to each other on a surface. These adherent cells are frequently embedded within a matrix of extracellular polysaccharide.
- Biofilms protect bacteria from host defences (e.g., antibodies), detergents and antibiotics. Biofilms also facilitate exchange of genetic material between bacterial cells leading to spread of antibiotic resistance among them.

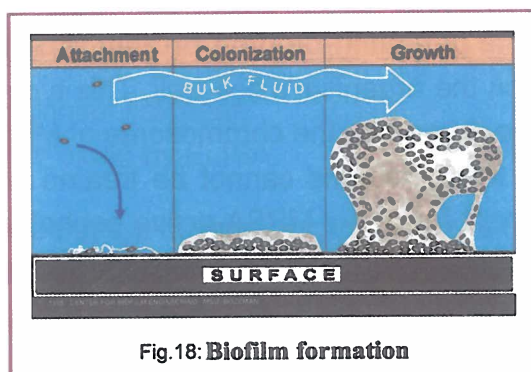


Fig.18: Biofilm formation

## Pathogenesis

*S. epidermidis* is part of the normal skin flora, and it is often attached to the upper layer of the skin (epidermis) or mucosa (carriage rate ~ 100%). Almost all infections are endogenous, but person-to-person transmission by contact may occur.

*S. epidermidis* is an opportunistic pathogen associated with **device-related infections** (e.g., catheter-related sepsis, prosthetic valve endocarditis, prosthetic joints and shunt infections), urinary tract and surgical wound infections.

## Laboratory diagnosis

*S. epidermidis* is diagnosed by its morphological and cultural characteristics. It is sensitive to novobiocin.

## Treatment

*S. epidermidis* infections are difficult to treat. This is because:

- The organism is often multi-resistant to antibiotics.
- The infections usually occur in prosthetic devices where the bacteria can sequester themselves in a biofilm.

## II- *S. saprophyticus*

### Morphology and cultural characteristics

*S. saprophyticus* is similar to *S. epidermidis*.

## Pathogenesis

*S. saprophyticus* may form part of the normal flora of human skin and mucosa of genitourinary tract. It may spread to urinary tract in colonized young sexually-active women causing urinary tract infections (honeymoon cystitis) (**endogenous infection**). This is due to the ability of the organism to adhere to uroepithelial cells.

## Laboratory diagnosis

*S. saprophyticus* is similar to *S. epidermidis* except in being novobiocin resistant.

## Treatment

Quinolones are the drugs of choice.

### MCQs:

- 1- The localized nature of *S. aureus* lesions is due to:
  - a- Adhesins
  - b- Protein A
  - c- Staphylocoagulase
  - d- Staphylokinase
  - e- Catalase
- 2- The following statements about *S. aureus* food poisoning are true **EXCEPT**:
  - a- It is caused by enterotoxins.
  - b- The source of contamination is usually a carrier.
  - c- The incubation period is 24-36 hours.
  - d- Food contains preformed toxin.
  - e- The responsible toxin acts as a superantigen.
- 3- MRSA isolates are treated empirically by:
  - a- Erythromycin
  - b- Vancomycin
  - c- Clindamycin
  - d- Cephalosporins
  - e- Tetracycline
- 4- The most important factor enabling *S. epidermidis* to colonize prosthetic devices is:
  - a- Production of coagulase
  - b- Resistance to many antibiotics
  - c- Production of glycocalyx
  - d- Production of exotoxin
  - e- Production of clumping factor



## STREPTOCOCCUS

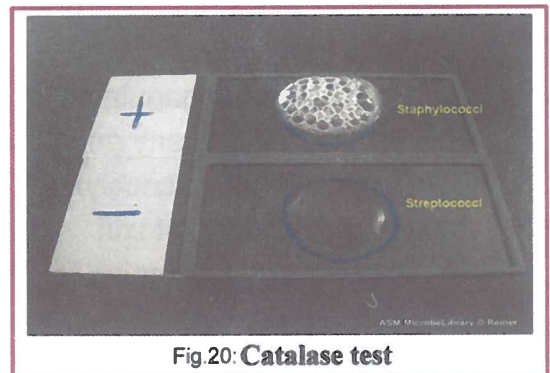
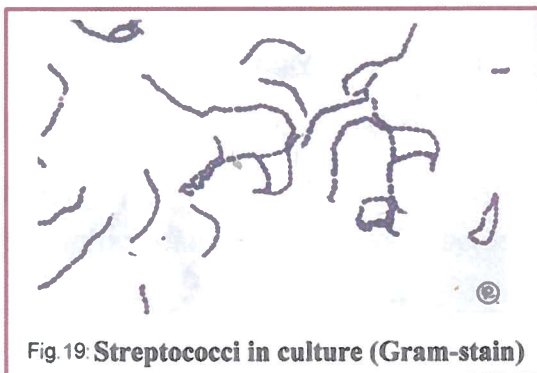
### ILOs:

By the end of this chapter the student should be able to:

- Describe the morphology and classify species of *Streptococcus*
- Define the growth requirements of *Streptococcus*
- Describe culture characteristics of beta and alpha haemolytic streptococci
- Outline and describe virulence factors of beta and alpha haemolytic streptococci
- Discuss the pathogenesis & list diseases of beta and alpha haemolytic streptococci
- Outline laboratory diagnosis of beta and alpha haemolytic streptococci
- Describe non-suppurative complications of *S. pyogenes* infections
- Outline treatment of infections caused by beta and alpha haemolytic streptococci
- State measures for prevention of streptococcal infections

### Characters of the genus *Streptococcus*

1. Gram-positive ovoid cocci, arranged in chains or pairs. (Fig.19)
2. Catalase negative: Catalase test is a key test for discriminating streptococci from the catalase-positive staphylococci. (Fig.20)
3. Growth requires enriched media containing blood or serum.



*Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus pneumoniae* are the most important species.

The medically significant streptococci may be conveniently divided on the basis of:

- Haemolysis on blood agar (complete haemolysis, beta; partial haemolysis, alpha; no haemolysis, gamma). (Fig.21)
- A group-specific carbohydrate antigen, according to which streptococci are classified into groups A to U (**Lancefield classification**). Antibodies against these group antigens are used for identification of streptococcal species.

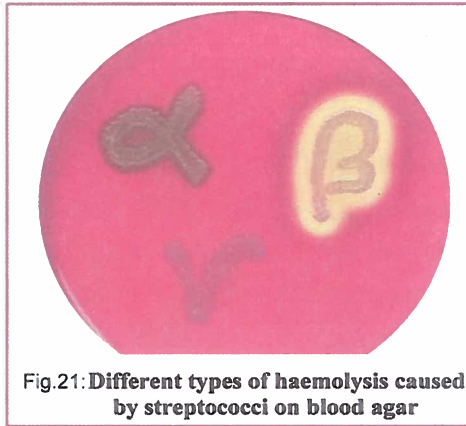


Fig.21: Different types of haemolysis caused by streptococci on blood agar

## Beta-Haemolytic Streptococci

### 1- *Streptococcus pyogenes* (Group A Streptococcus):

#### Morphology

*S. pyogenes* are Gram positive cocci in chains.

#### Cultural characters

- *S. pyogenes* produce beta-haemolysis on blood agar. (Fig22.)
- *S. pyogenes* growth is inhibited by bacitracin. (Fig.23)

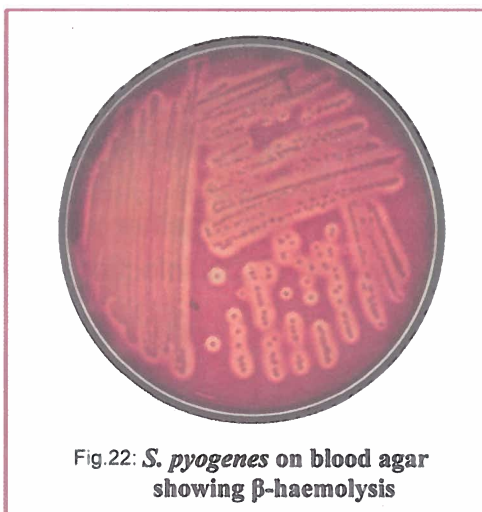


Fig.22: *S. pyogenes* on blood agar showing  $\beta$ -haemolysis

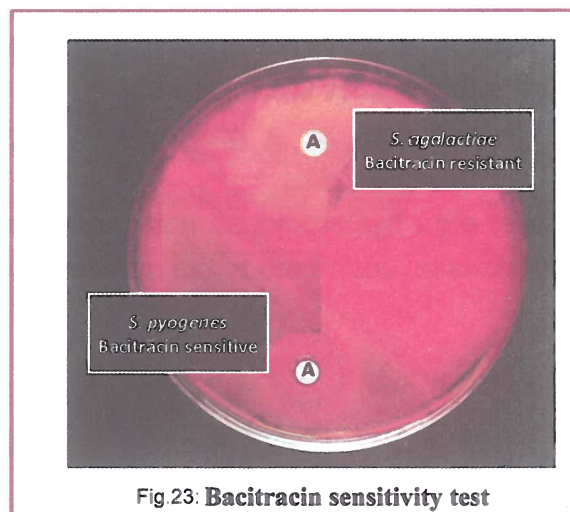


Fig.23: Bacitracin sensitivity test

## Virulence factors and pathogenesis

### I. Factors that mediate adherence (colonization):

1. **M protein:** It is a surface protein which enables the bacteria to colonize skin and to escape phagocytosis. It is the most important virulence factor. It is immunogenic and divides *S. pyogenes* into about 80 M serotypes.
2. **Fibronectin-binding protein (Protein F).**
3. **Lipoteichoic acids.**

### II. Factors that mediate invasion:

#### 1. Antiphagocytic factors:

- a- **M protein.**
- b- **C5a peptidase** breaks down C5a so that it no longer attracts phagocytes.
- c- **Hyaluronic acid capsule:** It is chemically similar to that of host connective tissue, therefore, it is not immunogenic. This allows the bacterium to hide its own antigens and to go unrecognized by its host.

#### 2. Invasins:

- a- **Streptokinase (fibrinolysin):** It activates plasminogen of human plasma into plasmin that digests fibrin and fibrinogen. It is ten times more potent than staphylokinase and is used for emergency therapy of myocardial infarction to remove blood clots.
- b- **Streptolysins (haemolysins):** These are two pore-forming toxins that lyse host cell membranes. Streptolysin O (oxygen labile) is a highly immunogenic protein and induces specific antibody formation (its detection is the basis for the anti-streptolysin O test). Streptolysin S (oxygen stable) is non-immunogenic.
- c- **Streptococcal pyrogenic exotoxins (SPE- A, B & C):** These toxins act as **superantigens** causing toxic shock syndrome, septicaemia, and necrotizing fasciitis.

In addition:

- Toxin A is an **erythrogenic** toxin that is responsible for the red rash characteristic of scarlet fever.
  - Toxin B acts as a **protease**. It contributes to the pathogenesis of necrotizing fasciitis.
- d- **Others:** e.g., hyaluronidase and nucleases. These enzymes, together with streptokinase, contribute to the spreading nature of streptococcal infections.



## *Streptococcus pyogenes* Infections

### I- Localized infections:

#### 1. Pharyngitis (sore throat, tonsillitis) (Fig.24)

- It is the **commonest** infection caused by *S. pyogenes*.
- It is characterized by pain, redness and swelling of posterior pharynx, accompanied by greyish white tonsillar exudate and fever.
- The organism is usually transmitted via respiratory droplets.

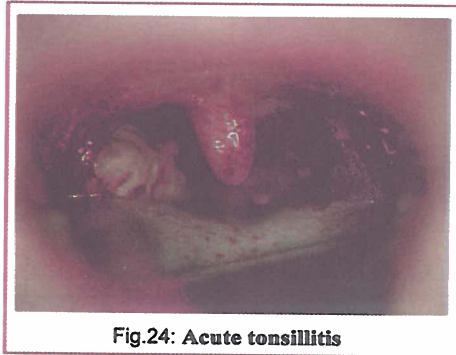


Fig.24: Acute tonsillitis

#### 2. Scarlet fever (Fig.25)

- It occurs when the streptococcal infection (especially pharyngitis) is caused by an erythrogenic toxin-producing *S. pyogenes*.
- It is characterized by development of scarlet red rash (sandpaper rash) and strawberry tongue.

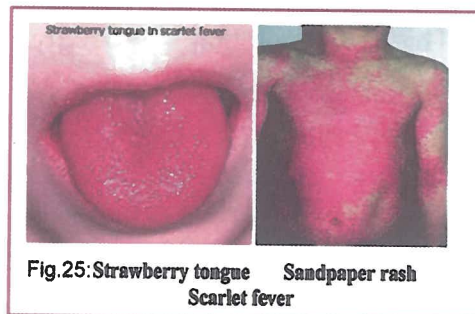


Fig.25: Strawberry tongue      Sandpaper rash  
Scarlet fever

#### 3. Skin and soft tissue infections

- **Impetigo (pyoderma):** It is a local infection of the superficial layers of the skin with blisters and denuded surface covered with crusts. (Fig.26)

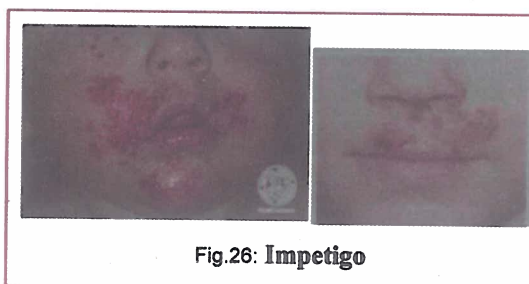


Fig.26: Impetigo

- **Cellulitis:** It is infection of the deep layers of the skin. (Fig. 27)
- **Erysipelas:** It is a form of cellulitis accompanied by fever and systemic toxicity. (Fig. 28)

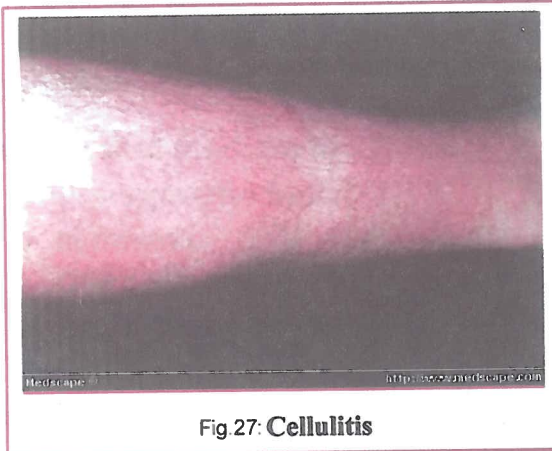


Fig 27: Cellulitis

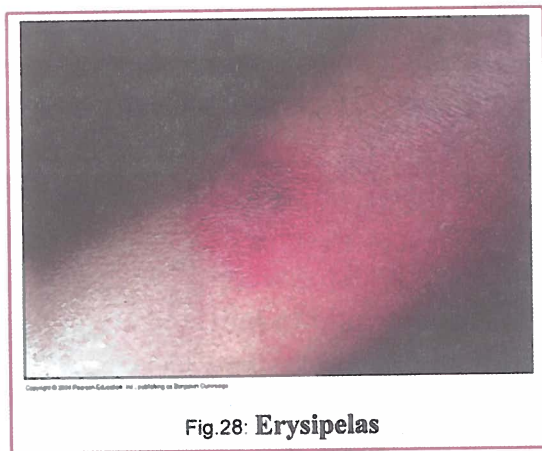


Fig.28: Erysipelas

## II- Invasive infections:

### 1- Puerperal fever:

- This is a life-threatening infection of the endometrium and surrounding structures complicating delivery or abortion.
- Septicaemia and toxic shock may occur.

### 2- Acute endocarditis:

This fatal condition can occur in individuals with normal or damaged heart valves.

### 3- Necrotizing fasciitis: (Fig.29)

- It is associated with severe tissue destruction particularly associated with SPE-B (protease).
- The destructive nature of this condition led to the term "flesh-eating bacteria".



Fig.29: Necrotizing fasciitis

#### 4- Toxic shock syndrome:

- This condition is mediated by the production of SPE- A, B & C.
- It often begins with skin wounds or minor traumas and rapidly deteriorates leading to **necrotizing** soft tissue infections.
- Shock, renal failure and acute respiratory distress syndrome are complications of the condition.

### Laboratory diagnosis

**A. Specimens** include throat swab, pus, blood (in invasive infections)...etc. (Fig.30)



Fig.30: Throat swab

#### B. Direct detection in clinical specimens

- 1- **Gram-stained smears** are useful only in cases of skin and soft tissue infections since *S. pyogenes* cannot be visually distinguished from the normal oral streptococcal flora. (Fig.31)

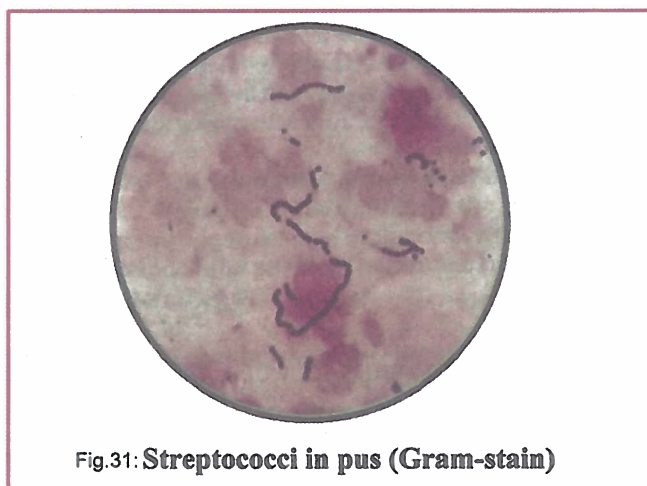


Fig.31: Streptococci in pus (Gram-stain)

- 2- **Detection of Lancefield group A streptococcal antigen** in throat swab by an agglutination test using group A antibody. (Fig.32)

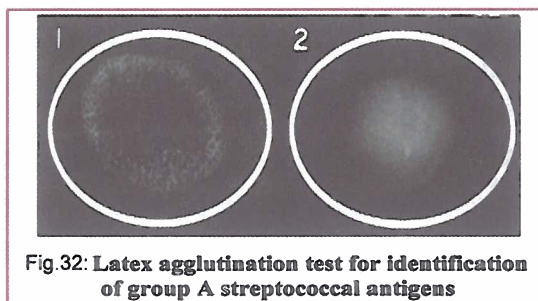


Fig.32: Latex agglutination test for identification of group A streptococcal antigens



### C. Cultivation

- Specimens other than the blood should be plated directly onto blood agar and incubated at 37°C.
- Blood samples should be cultivated by the blood culture technique. Subcultures are plated on blood agar.

### D. Identification

- After 24h incubation, the growth should be examined for colony morphology, Gram stain and catalase production:
  1. On blood agar: Colonies are surrounded by beta-haemolysis.
  2. Gram-stained film: Gram positive cocci in chains.
  3. Catalase test: negative.
- *S. pyogenes* can further be identified by:
  1. Growth inhibition by bacitracin (i.e. it is bacitracin-sensitive). (Fig.33)
  2. Agglutination by group A antibody.

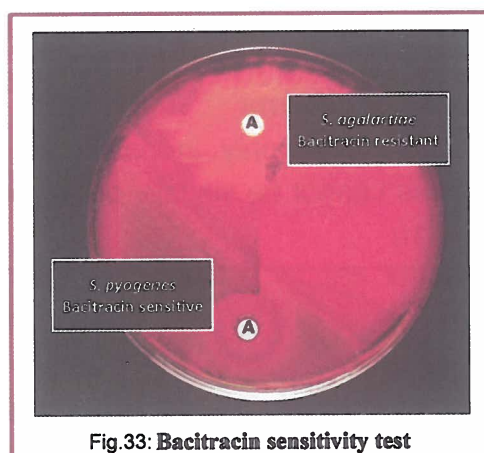


Fig.33: Bacitracin sensitivity test

## Post-Streptococcal Sequelae (Acute Rheumatic Fever and Acute Glomerulonephritis)

- These are non-suppurative inflammatory conditions which occur as a result of immunologic response to streptococcal antigens.
- They occur weeks following a local infection with *S. pyogenes*, and affect an organ that was not infected by the streptococci.
- Some streptococcal M serotypes are rheumatogenic and cause acute rheumatic fever (ARF), others are nephritogenic and cause acute glomerulonephritis (AGN).

### Acute rheumatic fever (ARF)

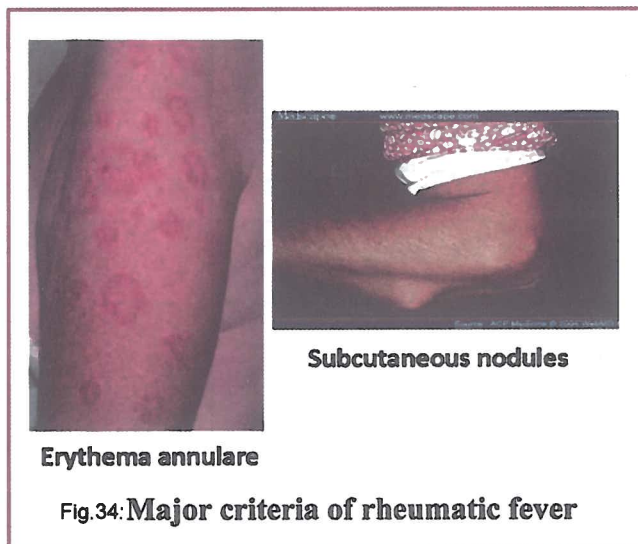
- ARF may affect any age (but mostly 4-30 years).
- It develops 2-3 weeks following streptococcal pharyngitis (but not skin infection).
- It is due to the formation of antibodies to streptococcal M protein, which cross-react with antigens of joints, heart and brain tissue (autoimmune reaction).
- The disease is characterized by fever, migrating poly-arthritis, carditis and chorea.

- The immunological process is exacerbated by recurrence of streptococcal pharyngitis leading to valvular damage. Therefore, long-term antibiotic prophylaxis using penicillin is recommended following a single attack.
- Rheumatic fever is preventable if the patient is treated within the first 10 days following onset of acute pharyngitis.

### Diagnosis of ARF:

No single test is diagnostic. Diagnosis is usually based on the modified Jones criteria. Diagnosis requires an evidence of recent *S. pyogenes* infection together with two major criteria, or one major and two minor criteria (listed below).

- **Evidence of recent streptococcal infection:** any of the following
  - 1- A history of acute tonsillitis (or scarlet fever).
  - 2- Positive throat swab culture for *S. pyogenes*.
  - 3- Elevation of antistreptolysin O (ASO) titre above 200 units. A titre of up to 200 units is considered normal.
- **Major criteria:** (1) Carditis. (2) Migratory polyarthritis. (3) Erythema annulare. (4) Subcutaneous nodules. (5) Chorea. (Fig.34)



- **Minor criteria:** (1) Elevated erythrocyte sedimentation rate, positive C-reactive protein or increased white cell count. (2) Fever. (3) Prior history of RF.

### Acute glomerulonephritis (AGN)

- AGN affects children more than adults.
- It develops one week mostly following skin infection rather than pharyngitis.

- It occurs due to deposition of antigen-antibody complexes on the glomerular basement membrane, provoking an inflammatory response that damages the kidney (type III hypersensitivity).
- It is characterized by hypertension, oedema of the face and ankles and smoky urine (due to RBCs in urine). Most patients recover completely and chronic renal failure rarely occurs.
- Reinfection with streptococci rarely leads to recurrence of AGN and antibiotic prophylaxis is unnecessary.
- Treatment of the streptococcal skin disease or pharyngitis does not prevent AGN.

### Diagnosis of AGN:

- There are elevated antibody titres for anti-DNase B.
- In glomerulonephritis following pyoderma or skin infection, the ASO titres are generally low. After throat infection (rare), the ASO titres may be higher.

**Table (1): Comparison between acute rheumatic fever & acute glomerulonephritis**

	ARF	AGN
<b>Pathogenesis</b>	Anti-M protein antibodies cross-react with epitopes on heart	Deposition of antigen-antibody complexes in the glomeruli
<b>Age</b>	Mostly between 4-30 years	Children more than adults
<b><i>S. pyogenes</i> strains</b>	Rheumatogenic	Nephritogenic
<b>Precipitating inf.</b>	Pharyngitis (but not skin infection)	Skin infections or pharyngitis
<b>Recurrence</b>	Common	Uncommon
<b>Chemoprophylaxis</b>	Essential	Unnecessary
<b>Sequelae</b>	Heart disease	Rarely, chronic renal failure
<b>Early treatment of precipitating inf.</b>	Prevents the condition	Does not prevent the condition
<b>Serological test</b>	ASO	Anti-DNAase, ASO (doubtful)

### Treatment and Prevention

- Penicillin is still uniformly effective in treatment of *S. pyogenes* infections.
- Long acting penicillin is used as a chemoprophylactic agent against recurrent *S. pyogenes* infections to prevent repeated rheumatic attacks.
- Erythromycin is used as an alternative for penicillin-allergic patients.



## 2- *Streptococcus agalactiae* (Group B *Streptococcus*; GBS):

- *S. agalactiae* is beta-haemolytic, bacitracin-resistant streptococci with a polysaccharide capsule.
- About 25% of pregnant women are vaginal carriers for GBS.
- GBS infections are acquired by neonates at the time of birth; therefore, GBS are important aetiological agents of infections during the first two months of life.
- **Diseases caused by *S. agalactiae*:**
  - 1- Neonatal sepsis which may manifest as pneumonia, septicaemia and meningitis.
  - 2- Serious infections in adults e.g., pneumonia and endocarditis particularly in cancer and diabetic patients.
- **Prevention:** Routine screening for *S. agalactiae* in pregnant women at the end of the 3<sup>rd</sup> trimester. Colonized mothers are given ampicillin during delivery (intrapartum) to reduce neonatal sepsis.

## Alpha-Haemolytic Streptococci

### 1- Viridans Streptococci: (Fig.35)

- They are normal inhabitants of the oral cavity, gastrointestinal and female genital tracts.
- Viridans streptococci play a significant role in **dental caries**. (Fig.36)
- They are responsible for nearly 50% of all cases of **subacute bacterial endocarditis (SBE)**. (Fig.37)



Fig.35: Viridans streptococci on blood agar showing  $\alpha$ -haemolysis



Fig 36: Dental caries

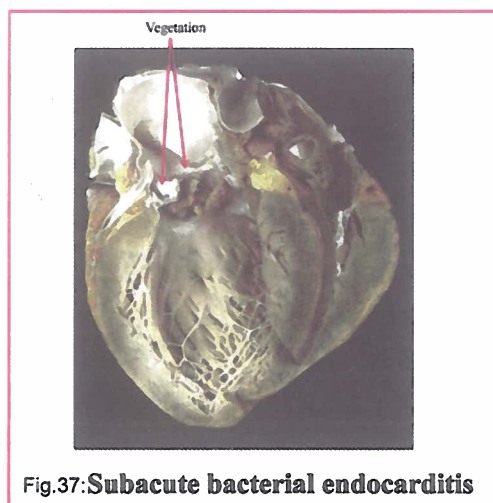


Fig.37:Subacute bacterial endocarditis

- SBE may occur when dental manipulations or trauma to mucosa of upper respiratory tract, e.g. tonsillectomy, lead to bacteraemia.
- The organisms can adhere to cardiac valves, especially in people with underlying valvular disease (prosthetic valve, deformed heart valve e.g., rheumatic or congenital heart, ..... etc.) resulting in endocarditis.

### Laboratory diagnosis of SBE

- It is carried out by the **blood culture technique** with subculture on blood agar.
- The isolated organism should be discriminated from *S. pneumoniae* (Table 2).

### Treatment

- Viridans streptococci are relatively resistant to penicillin.
- The use of the synergistic combination of penicillin and gentamicin in life-threatening infections, such as endocarditis, is essential.

### Prevention

A single large dose of ampicillin or amoxicillin should be given to patients with abnormal heart valves prior to dental procedures or oral surgery to prevent endocarditis.

## 2- *Streptococcus pneumoniae* (Pneumococci):

### Morphology

Gram-positive, lancet-shaped, capsulated, diplococci. (Fig.38)

### Culture

On blood agar, colonies show  $\alpha$ -haemolysis.



Fig.38: Pneumococci

## Virulence factors

The most important virulence factor is the **polysaccharide capsule**:

- It is antiphagocytic.
- It divides the organism into about 90 antigenically different serotypes.
- The capsule reacts with the specific antibody and can be seen under the microscope to swell. This is the basis of the **quellung test** which is used to identify the pneumococcal serotypes. (Fig.39)

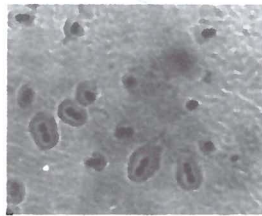


Fig.39: Quellung reaction

## Pathogenesis

*S. pneumoniae* is carried in the pharynx of about one third of adults. It is not considered highly communicable; therefore, pneumococcal infections are mostly **endogenous**, although exogenous infections by respiratory droplets may also occur.

Individuals at risk include the alcoholics, post-splenectomy, immunosuppressed, infants and the elderly. Pneumococci cause:

- **Pneumonia, meningitis and otitis media** which are the three major diseases.
- Sinusitis, conjunctivitis, endocarditis and septic pericarditis may also occur.

## Laboratory diagnosis

**A- Specimens** include sputum (characteristically blood-tinged "rusty" sputum), CSF, ear or eye discharge and blood (in cases of bacteraemia accompanying pneumonia, meningitis and endocarditis).

### B- Direct detection

- 1- **Gram-stained smear:** Gram-positive, capsulated, diplococci.

The capsule appears as an unstained zone around the organism. (Fig.40)

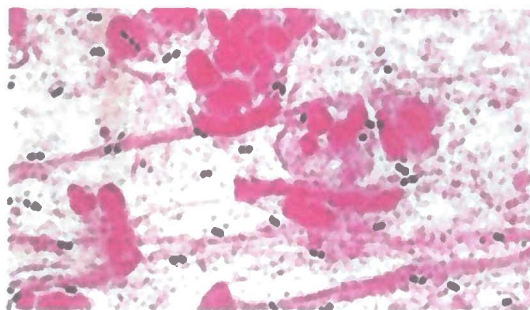


Fig.40: Pneumococci in tissue (Gram-stain)



## 2- Quellung test.

## 3- Detection of capsular polysaccharide antigen in CSF by means of latex agglutination test.

**C-Cultivation**

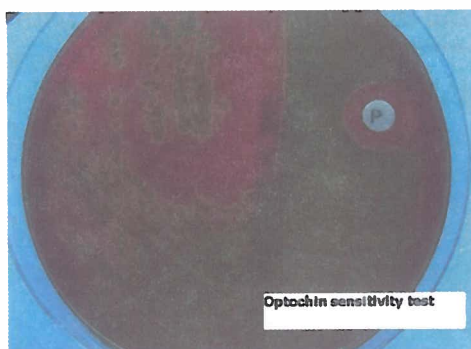
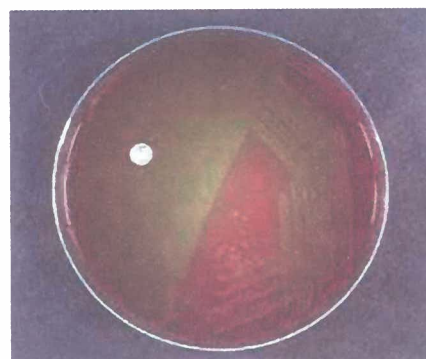
- Specimens other than the blood should be plated directly on blood agar and incubated at 37°C.
- Blood samples should be cultivated by the blood culture technique.

**D-Identification**

- 1- After 24 h incubation, the growth should be examined for colony morphology, catalase test and Gram stain (see before).
- 2- Colonies of viridans streptococci (non-pathogenic) are also encountered on blood agar while examining sputum for *S. pneumoniae* (pathogenic). Confusion occurs due to similarity in microscopic and colony morphology. Discrimination must be done according to table (2):

Table (2): Discrimination between *S. pneumoniae* and viridans streptococci

Test	<i>S. pneumoniae</i>	Viridans strept.
Growth inhibition by optochin (Fig.41)	+	-
Solubility of colonies in bile (Fig.42)	+	-
Capsular Ag detection (latex agglutination)	+	-
Quellung test	+	-

Fig.41: Optochin sensitivity test  
Pneumococci (sensitive)Optochin sensitivity test  
Viridans streptococci (resistant)

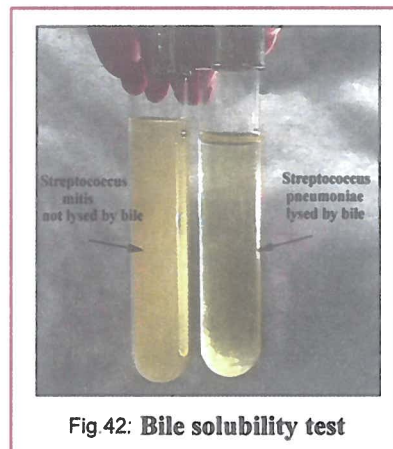


Fig.42: Bile solubility test

## Treatment

Third generation cephalosporins (e.g. ceftriaxone) are the drugs of choice. Penicillin resistance in pneumococci has been reported. Vancomycin is the drug of choice for penicillin resistant pneumococci.

## Prevention

- **Capsular polysaccharide vaccine:** It contains antigens from the most common 23 pneumococcal serotypes. Anti-capsular antibody confers type specific protection. The vaccine is used after splenectomy, in elderly and immunosuppressed patients. It is not effective in children less than 2 years of age who respond poorly to polysaccharide (thymus independent) antigen.
- **Protein conjugate vaccine:** It contains the capsular polysaccharide of the 13 most common pneumococcal serotypes conjugated to a protein carrier that makes the vaccine more effective in children less than 2 years of age. It is recommended to be given in 4 doses (at 2, 4, 6 months and 12-15 months).

## MCQs:

- 1- The following are virulence factors for *S. pyogenes* **EXCEPT**:
  - a- Fibronectin binding protein
  - b- M protein
  - c- Hyaluronic acid capsule
  - d- Fibrinolysin
  - e- Coagulase
  
- 2- Acute rheumatic fever:
  - a- Is diagnosed by elevated anti-DNAse antibodies
  - b- Usually follows streptococcal skin infections
  - c- Is caused by streptococcal invasion of the cardiac valves
  - d- Should be followed by chemoprophylaxis to prevent further attacks
  - e- Develops 3-6 months following an acute streptococcal disease
  
- 3- Which of the following procedures is most likely to reduce the incidence of group B streptococcal sepsis in infants:
  - a- Intrapartum antibiotic treatment
  - b- Use of a polysaccharide vaccine
  - c- Screening of pregnant females in the first trimester
  - d- Identification of possible high risk births
  - e- Antibiotic treatment of the newborn
  
- 4- After extraction of a tooth, a student with history of congenital heart disease was diagnosed as having subacute bacterial endocarditis. The most likely organism causing this infection is:
  - a- *Staphylococcus aureus*
  - b- *Staphylococcus epidermidis*
  - c- *Streptococcus pneumoniae*
  - d- Viridans streptococci
  - e- Enterococci
  
- 5- If a quellung test was done on the following bacterial isolates, which one would you expect to be positive?
  - a- *S. pneumoniae*
  - b- *S. pyogenes*
  - c- *S. aureus*
  - d- Viridans streptococci
  - e- *S. epidermidis*



## ENTEROCOCCUS

### ILOs:

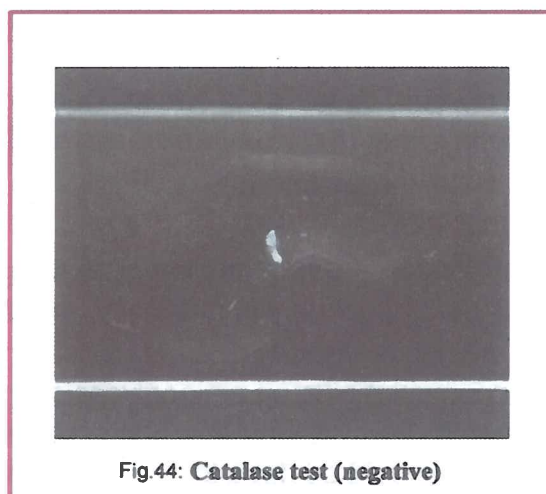
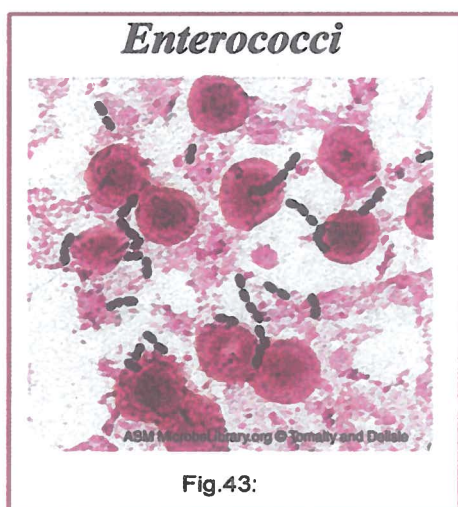
By the end of this chapter the student should be able to:

- Describe morphology and list species of *Enterococcus*
- Describe culture characteristics & growth requirements of species of *Enterococcus*
- List infections caused by enterococci
- Outline treatment and recognize the antibiotic susceptibility problems related to enterococci

### Characters of the genus *Enterococcus*

Enterococci are similar to streptococci in the following:

1. They are Gram-positive cocci that occur in pairs or in short chains. (Fig.43)
2. They are facultative anaerobes.
3. They are catalase negative. (Fig.44)
4. Most strains react with the streptococcal Lancefield group D antiserum.



Enterococci differ from streptococci in being able to:

1. Grow at 45°C.
2. Grow in broth containing 6.5% NaCl (salt tolerant).
3. Tolerate bile salts. This allows survival of enterococci in bowel and gall bladder.
4. Hydrolyze the polysaccharide esculin, producing black colonies on esculin-containing media. (Fig.45)

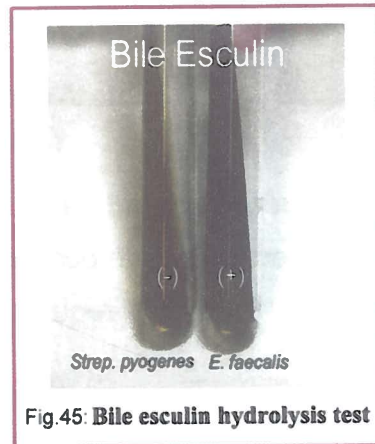


Fig.45: Bile esculin hydrolysis test

These organisms are found normally in the human intestine so they are used as an indicator of faecal pollution of water.

The common species are *Enterococcus faecalis* and *Enterococcus faecium*.

### Infections caused by enterococci

1. Urinary tract infections are the most common
2. Intra-abdominal or pelvic wound infections
3. Bacteraemia
4. Endocarditis
5. Abscesses, meningitis, peritonitis, osteomyelitis and wound infection

*Enterococcus faecalis* is most commonly isolated from healthcare-associated infections rather than community-acquired infections.

### Treatment

- Enterococci are frequently resistant to antibiotics.
- They are absolutely (intrinsically) resistant to cephalosporins and clindamycin.
- A synergic combination of a cell wall-active drug (penicillin, ampicillin, or vancomycin) plus an aminoglycoside (gentamicin or streptomycin) should be used for serious infections like bacteraemia, endocarditis or meningitis. Susceptibility testing should be done to select the appropriate combination for synergy.

### MCQs:

- 1- Enterococci differ from streptococci in the following **EXCEPT**:
  - a- They can grow at 45°C.
  - b- They are salt tolerant.
  - c- They tolerate bile salts
  - d- They hydrolyze the polysaccharide esculin.
  - e- They are catalase negative

## NEISSERIA

### ILOs:

**By the end of this chapter the student should be able to:**

- Describe morphology and classify species of the *Neisseria* genus
- Describe culture characteristics & growth requirements of pathogenic *Neisseria* species
- List and outline virulence factors of pathogenic *Neisseria* species
- Discuss pathogenesis & list diseases of pathogenic *Neisseria* species
- Outline laboratory diagnosis of pathogenic *Neisseria* species
- Outline treatment of diseases of pathogenic *Neisseria* species
- Describe measures for prevention of diseases of pathogenic *Neisseria* species
- State morphology and diseases caused by *Moraxella* species

### Characters of the genus *Neisseria*

1. Gram-negative cocci arranged in pairs with the adjacent sides flattened to give the characteristic kidney or coffee bean shape. (Fig.46,47)
2. Aerobic.
3. Oxidase-positive (key test for the *genus Neisseria*). (Fig.48)

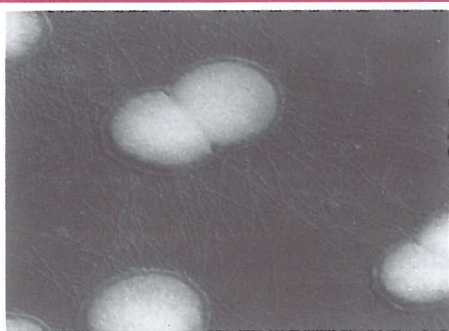


Fig.46 : *Neisseria* (diplococci)

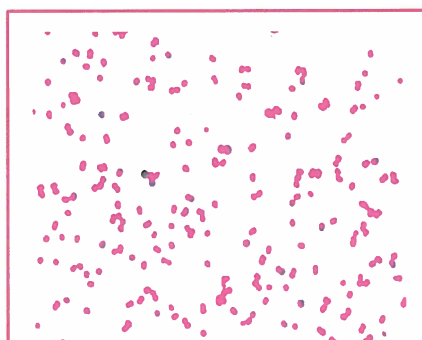


Fig.47 : *Neisseria* in culture (Gram-stain)

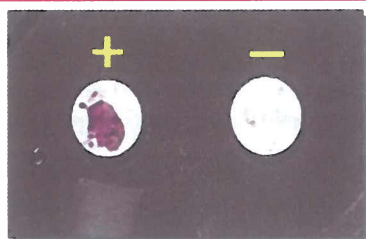


Fig.48 : Oxidase test



The genus includes:

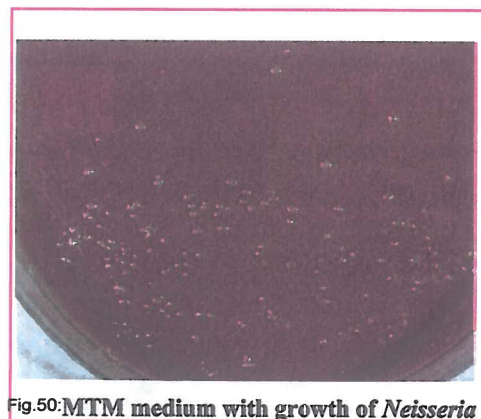
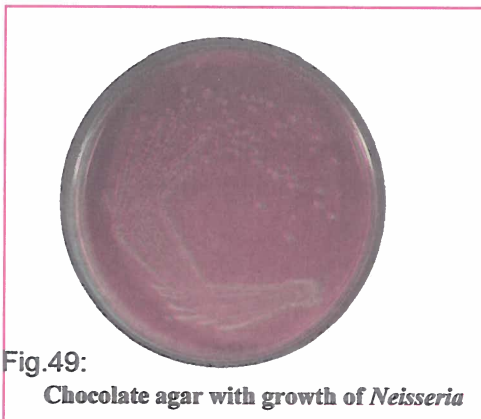
- **Many commensals:** e.g. *N. lactamica* and *N. sicca* that are inhabitants of oro- and nasopharynx of healthy individuals. They rarely cause diseases.
- **Two important human pathogens:**
  1. *Neisseria gonorrhoeae* (gonococci)
  2. *Neisseria meningitidis* (meningococci).

## *Neisseria gonorrhoeae*

**Morphology** (mentioned above)

### Cultural characters

- *N. gonorrhoeae* is the most fastidious neisseria species.
- It requires an enriched medium like chocolate agar but it does not grow on blood agar.(Fig.49)
- Modified Thayer-Martin (MTM) medium, which is chocolate agar that is rendered selective by adding certain antibiotics, allows easier isolation of the organism from specimens contaminated by other microbes.(Fig.50)
- Cultures should be incubated in a humid atmosphere with 5-10 % CO<sub>2</sub> at 37°C.



### Biochemical reactions

- *N. gonorrhoeae* is oxidase positive.
- *N. gonorrhoeae* utilizes glucose only with production of acid only.

### Virulence factors

1. Pili: mediate attachment to epithelial cells.
2. Outer membrane proteins which include:
  - a- Adherence proteins (Opa proteins): they get their name from the opaque appearance they give to colonies. They assist binding to epithelial cells.
  - b- Porin proteins: they promote intracellular survival inside phagocytes by:
    - preventing fusion of phagosomes and lysosomes.
    - suppressing the oxidative burst.
3. Lipooligosaccharides (LOS): a modified endotoxin that elicits an inflammatory response.

4. IgA protease: inactivates secretory IgA leading to more adherence to and colonization of the mucosa.

### Diseases Caused by *N. gonorrhoeae*

#### I. Gonorrhoea:

- **Pathogenesis of gonorrhoea:**

- Gonorrhoea is a sexually-acquired venereal disease primarily localized to mucosal surface.
- Gonococci cannot survive dryness, so intimate contact is necessary for transmission.
- Pili, Opa and IgA protease allow adherence and colonization to mucosa.
- Endocytosis is followed by intracellular growth that causes destruction of epithelial cells.
- Porin proteins and catalase allow gonococci to survive inside polymorph-nuclear leucocytes.

- **Manifestations:**

- **Men:** Gonococci infect the urethra leading to acute urethritis with dysuria and purulent discharge.(Fig.51)
- **Women:** Gonococci infect the cervix (not vagina), urethra, vulva and rectum leading to dysuria and cervicitis with a purulent cervical discharge. About half of infections in women, however, are asymptomatic and may contribute to persistence and spread of gonorrhoea.(Fig.52)

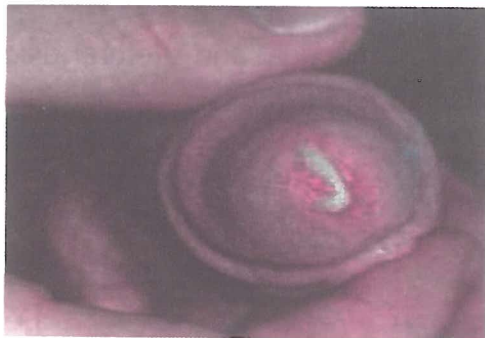


Fig.51: Urethral discharge  
Gonorrhoea



Fig.52: Cervical Discharge  
Gonorrhoea

- **Consequences of gonorrhoea:**

- **Men:** The organism may spread to the prostate, bladder and epididymis causing inflammation and swelling.
- **Women:** Ascending infection may occur leading to endometritis and salpingitis (pelvic inflammatory disease; PID). It manifests as fever and lower abdominal pain and may lead to ectopic pregnancy or sterility.
- **Disseminated gonococcal infection (DGI) or gonococcaemia:**
  - It occurs more in females (especially the pregnant) and individuals with defect of the terminal complement components.
  - It may manifest as arthritis accompanied with skin rash, meningitis or endocarditis.(Fig.53)



- DGI may result in disseminated intravascular coagulation (DIC) and shock due to the LOS endotoxin.



Fig.53: Gonococcaemia (DGI) skin rash

- II. **Neonatal conjunctivitis (ophthalmia neonatorum):** It is an acute conjunctivitis in infants born to mothers with gonorrhoea. The eyes become infected at the time of delivery, and -if untreated- can lead to blindness. (Fig.54)



Fig.54: Ophthalmia neonatorum

- III. **Vulvovaginitis:** Infection of vulva and vagina in young girls due to sexual abuse.

### Laboratory diagnosis of gonorrhoea

- A. **Specimens:** urethral discharge from men and cervical and urethral discharge from women. Other specimens, e.g., synovial fluid in case of arthritis may be examined.

B. **Direct detection**

1- **Gram-stained smears:**

- In men, the finding of Gram-negative diplococci within some polymorph-nuclear leucocytes in urethral discharge is sufficient for diagnosis. (Fig.55)

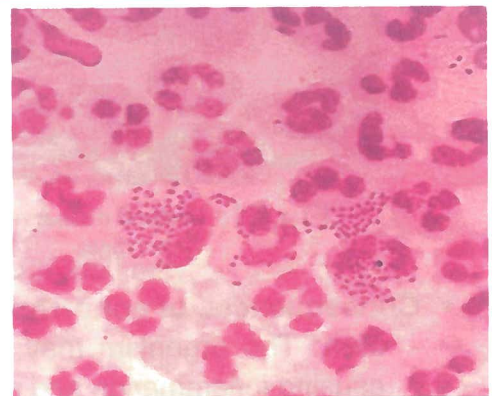


Fig.55: Pathogenic *Neisseria* in pus (Gram-stain)

- In women, the use of the Gram-stained smear alone can be difficult to interpret. They can be falsely positive because of the presence of Gram-negative diplococci in the normal flora, and can be falsely negative because of the inability to see the small number of gonococci. Therefore, cultures of cervical specimens should be done.
- 2- A nucleic acid probe test detects gonococcal nucleic acid in specimens.



**C. Cultivation:** The specimens are plated onto chocolate agar and MTM medium. Cultures should be incubated with 5-10% CO<sub>2</sub>.

#### D. Identification

- 1- Colonies should be examined by Gram stain and oxidase test. Colonies showing Gram-negative diplococci and positive oxidase test are considered *Neisseria*.
- 2- The organism is identified as *N. gonorrhoeae* by:
  - a. Glucose utilization with production of acid.
  - b. Nucleic acid probe.

#### Treatment

- Third generation cephalosporins (e.g. ceftriaxone) or spectinomycin is now recommended for treatment of gonococcal infections.
- Although *N. gonorrhoeae* is frequently resistant to tetracycline, this agent or azithromycin should be given due to the concurrent *Chlamydia* infection.

#### Prevention

1. Chemoprophylaxis in the form of erythromycin eye ointment is essential to prevent gonococcal neonatal conjunctivitis.
2. The use of condoms and the proper treatment of the symptomatic patients and their sexual partners is recommended.

N.B.: Repeated gonococcal infections may occur although gonococcal antibodies are produced. This is due to:

- a) High antigenic variations of gonococcal pili.
- b) Superficial nature of the infection.
- c) Production of IgA protease.

### *Neisseria meningitidis*

**Morphology** (as mentioned previously)

#### Cultural characters

*N. meningitidis* is similar in its cultural characters to *N. gonorrhoeae* except in its ability to grow on blood agar because it is less fastidious.

#### Biochemical reactions

- *N. meningitidis* is oxidase positive.
- *N. meningitidis* utilizes glucose and maltose with production of acid only.

## Virulence factors

1. The polysaccharide capsule, with its antiphagocytic action, represents the most important virulence factor. The capsule classifies meningococci into at least 13 serogroups.
2. LPS is responsible for the endotoxin effect of meningococcal infections.
3. Pili are responsible for attachment to nasopharyngeal mucosal cells.
4. IgA protease inactivates secretory IgA.

## Diseases Caused by *N. meningitidis* (Meningitis and Meningococcaemia)

The organism causes 20% of all cases of meningitis which may occur sporadically or in epidemic form (**epidemic cerebrospinal meningitis**). The most common serogroups causing disease are A, B, C, Y, and W135. **Serogroup A** accounts for most meningococcal epidemics especially in **Africa** and some parts of **Asia**.

## Pathogenesis

*N. meningitidis* is normally carried in the nasopharynx in 5-30% of healthy population. This carrier state increases immediately before and during epidemics. Infection is transmitted by **droplets** from carriers and cases.

- **Pili** allow the attachment of the organism to the mucosal epithelium of the nasopharynx and together with **IgA protease** establish bacterial colonization.
- Endocytosis takes place and a slight local inflammation (sore throat) occurs.
- The virulence of meningococci is primarily due to the invasive capacity of the **capsulated** organism.
- The organism enters the bloodstream (meningococcaemia). From the blood, the organism may settle in different parts of the body. Localization of organisms in the meninges leads to meningitis and cerebral oedema.

## Clinical manifestations

- The disease manifests as sudden severe headache, projectile vomiting and stiff neck. It may progress rapidly to coma and death or resolve with permanent neurological complications e.g. deafness, speech disability and paralysis.
- *N. meningitidis* may localize also in the joints or endocardium leading to arthritis or endocarditis. Skin rash usually occurs with meningococcaemia due to the **endotoxin** action of the LPS. In fulminant infections, DIC occurs which leads to shock and may end in death. (Fig.56)



Fig.57: Meningococcaemia (purpura)

- Particularly susceptible hosts include: a) children under the age of 3 years because they fail to make antibody against the antiphagocytic capsule (T1 antigen), and b) individuals having defect in the terminal complement components.

## Laboratory diagnosis

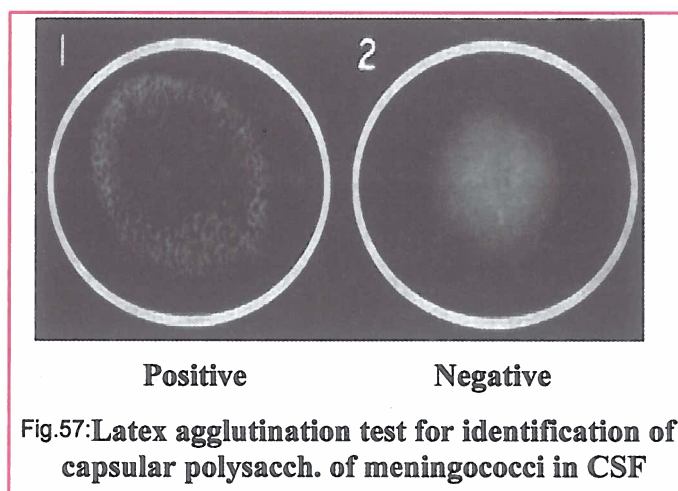
Meningitis is a **medical emergency**, so diagnosis must be rapid and precise.

**A. Specimens:** CSF, blood and aspirates from joint fluid.

- **CSF examination:** CSF shows characteristics of bacterial (septic) meningitis as follows:
  - Physically: CSF is cloudy and under tension.
  - Cytologically: High leucocytic count (200-20,000/ml) with predominant neutrophils.
  - Chemically: Reduced glucose level and elevated protein level.

## B. Direct detection

- 1- **Gram-stained smears** prepared from CSF show Gram-negative intracellular diplococci.
- 2- Direct detection of meningococcal antigen in CSF by latex agglutination or fluorescent antibody test.(Fig.57)



The result is essential to direct the antimicrobial therapy and must be reported within one hour to the treating physician. Definitive diagnosis by culture is essential to apply the required infection control measures.

## C. Cultivation

- 1- Specimens other than the blood are plated onto chocolate agar, blood agar and MTM medium. Cultures should be incubated in a humid atmosphere with 5-10% CO<sub>2</sub> at 37°C.



- 2- Blood samples should be cultivated by the blood culture technique. Subcultures are plated on chocolate or blood agar and incubated as mentioned above.

#### D. Identification

- 1- Colonies should be examined by Gram stain and oxidase test. Colonies showing Gram-negative diplococci and positive oxidase test are considered *Neisseria*.
- 2- The organism is identified as *N. meningitidis* by:
  - a. Utilization of glucose and maltose with production of acid only.
  - b. Detection of specific antigens by latex agglutination or immunofluorescence.
  - c. Nucleic acid probe.

### Treatment

- Third-generation cephalosporins such as ceftriaxone or cefotaxime are currently the drugs of choice for empiric therapy.
- **Penicillin G** has long been the treatment of choice for meningococcal infections because of its high anti-meningococcal activity and good penetration into CSF. Strains resistant to penicillin have rarely emerged.

### Prevention

#### A- Vaccination

- **Capsular polysaccharide vaccines:**
  - Bivalent including serogroups A and C.
  - Quadrivalent including serogroups A, C, W-135 and Y.
- Group B capsular substance is not yet included in the vaccine. It is poorly immunogenic because of its similarity to sialic acid found in human tissues.
- **Protein conjugate vaccines** are given to children less than two years old who respond poorly to polysaccharide (thymus independent) antigens.
- The vaccine provides 80-90% protection.
- Induced immunity lasts for about 3-5 years.
- Vaccination is particularly important to groups at risk e.g. military recruits, school children, college students as well as travelers to certain parts of the world (e.g. pilgrims).

**B- Chemoprophylaxis** is recommended for close contacts, e.g. healthcare and laboratory workers, household contacts and in outbreaks. The antibiotic recommended is either rifampin for 2 days orally or one ceftriaxone injection.

**C- Droplet precautions** including use of masks is recommended for the cases and contacts.

***Moraxella (Branhamella) catarrhalis***

- *Moraxella* is a genus in the family *Neisseriaceae*.
- It is an oxidase positive, Gram negative organism that resembles *neisseriae*.
- It may occur as normal flora of the nasopharynx in humans.
- It causes respiratory tract infections including bronchitis, pneumonia, otitis media and sinusitis. It may cause endocarditis and meningitis in the elderly patients.

**MCQs:**

- 1- All the following are characters of the genus *Neisseria* EXCEPT:
  - a- Gram negative diplococci
  - b- Kidney shaped
  - c- Aerobic
  - d- Oxidase negative
  - e- Including commensals and pathogenic species
- 2- *N. gonorrhoeae* is a fastidious pathogen found in sites often contaminated with normal flora. The best medium for isolation is:
  - a- Blood agar
  - b- Loeffler's medium
  - c- Thayer-Martin medium
  - d- Thiosulphate citrate bile salts sucrose medium
  - e- Lowenstein Jensen medium
- 3- In adult females, gonococci infect the following sites EXCEPT:
  - a- The cervix
  - b- The vagina
  - c- Urethra
  - d- Vulva
  - e- Rectum
- 4- The most important virulence factor in *N. meningitidis* is:
  - a- IgA protease
  - b- Outer membrane protein
  - c- Pili
  - d- The polysaccharide capsule
  - e- Lipid A

## NON-SPORE-FORMING GRAM-POSITIVE BACILLI

### ILOs:

By the end of this chapter the student should be able to:

- Describe morphology and identify species of the *Corynebacterium* genus
- Describe culture characteristics and growth requirements of *Corynebacterium diphtheriae*
- Point out the morphological and cultural features which differentiate *Listeria* from corynebacteria
- List and outline important virulence factors of *Corynebacterium diphtheriae* and *Listeria monocytogenes*
- Discuss pathogenesis and diseases caused by *Corynebacterium diphtheriae* and *Listeria monocytogenes*
- Outline laboratory diagnostic methods for diphtheria
- Outline the treatment and describe the prevention of diphtheria
- Describe morphology of *Propionobacterium* and *Lactobacillus*
- Recognize *Propionobacterium* and *Lactobacillus* as members of the normal flora, state their metabolic products and their diseases
- Define probiotics and relate the role of *Lactobacillus* regarding this aspect

The Gram-positive non-spore-forming rods are subdivided into:

- Aerobic genera (*Corynebacterium* and *Listeria*).
- Anaerobic genera (*Propionibacterium* and *Lactobacilli*).



## CORYNEBACTERIUM

### Characters of the genus *Corynebacterium*

- 1- Gram-positive bacilli with a characteristic club-shape, hence the genus name (club = Coryne).
- 2- Aerobic.

The genus comprises:

- *Corynebacterium diphtheriae*, the causative agent of diphtheria.
- Many commensal species of skin and mucous membranes. They are termed "diphtheroids" because they resemble *C. diphtheriae* in morphology. They rarely cause disease in immunocompromised individuals. (Fig.58)

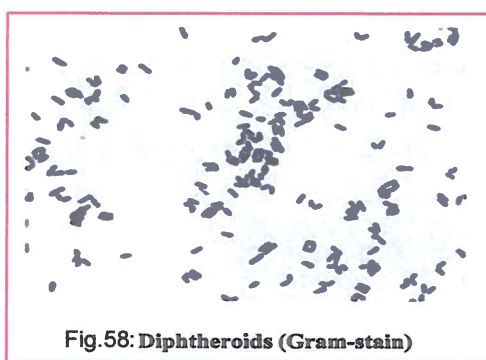


Fig.58: Diphtheroids (Gram-stain)

### *Corynebacterium diphtheriae*

#### Morphology

- Club-shaped pleomorphic Gram-positive bacilli.
- Arranged at acute angles or parallel to each other (Chinese letters appearance). (Fig.59)
- The bacilli appear beaded when stained by methylene blue stain due to metachromatic granules. (Fig.60)

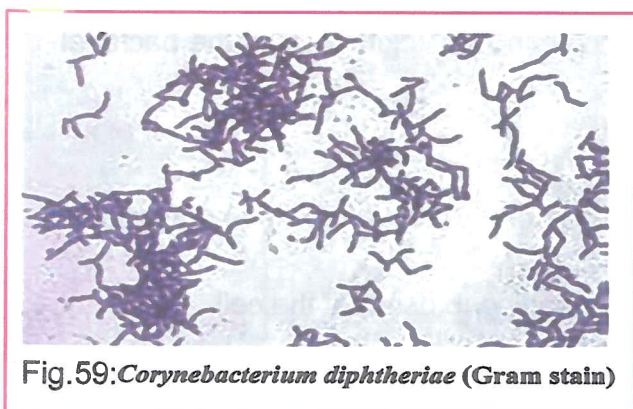


Fig.59: *Corynebacterium diphtheriae* (Gram stain)

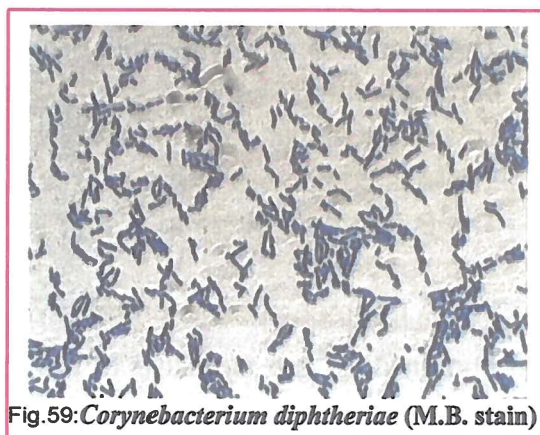


Fig.59: *Corynebacterium diphtheriae* (M.B. stain)

## Cultural characters

*C. diphtheriae* can grow on:

- Enriched media e.g., blood agar and Loeffler's serum media. (Fig.61)

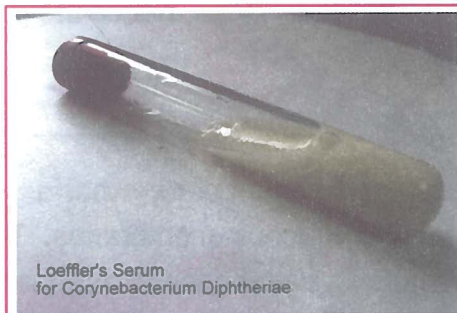


Fig.61: Loeffler's serum for culture of *C. diphtheriae*

- Selective differential media containing tellurite (e.g. blood tellurite agar) which are used to isolate *C. diphtheriae* from sites where bacterial flora predominates. *Corynebacterium* colonies are gray/black on tellurite agar. (Fig.62)

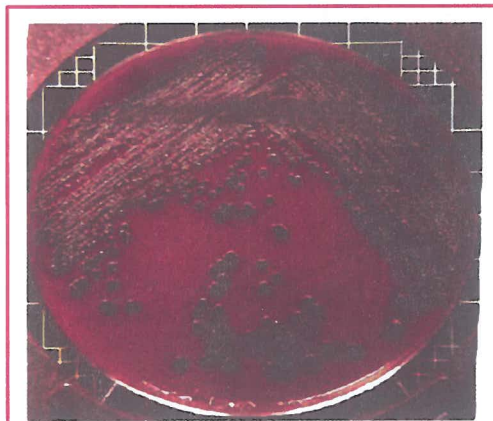


Fig.62: *C. diphtheriae* on blood tellurite

## Virulence factors

- The virulence factor of *C. diphtheriae* is **exotoxin**.
- Toxin production depends on:
  - 1- The presence of a lysogenic prophage carrying the toxin gene in the bacterial chromosome.
  - 2- Low extracellular concentration of iron.
- The toxin is composed of A and B subunits:
  - The B fragment binds to a specific receptor on susceptible cells and mediates entry of fragment A.
  - The A fragment inhibits protein synthesis leading to death of the cell.
- All human cells are sensitive to the toxin especially the cardiac muscle and the cranial and peripheral nerves.

## Diphtheria

### Pathogenesis

- Diphtheria is transmitted by droplets, from a case or a carrier.
- The incubation period of diphtheria is 2-5 days.
- Diphtheria involves both local and systemic pathology:
  1. **Local pseudomembrane:** It develops in the upper respiratory tract (tonsils, pharynx, larynx, and/or nose). It is an adherent membrane composed of mucosal cell debris, infection products and fibrinous exudates. (Fig.63)



Fig.63: Pharyngeal pseudomembrane in a case of diphtheria

2. **Toxaemia:** The diphtheria bacilli do not invade tissues. They produce the toxin that is absorbed and disseminated through the blood to the susceptible tissues.

### Clinical manifestations

- The commonest form of diphtheria is the pharyngeal (or tonsillar) diphtheria.
- Cervical lymphadenitis (bull neck) is one of the initial symptoms. (Fig.64)

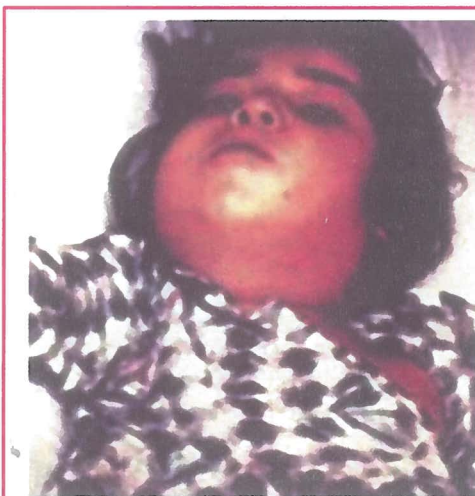


Fig.64: Bull neck in a case of diphtheria



- It is usually associated with severe toxæmia. The patient appears quite toxic with low-grade fever.
- There are 3 main complications:
  1. Extension of the membrane into the larynx causing airway obstruction.
  2. Myocarditis leading to heart failure.
  3. Nerve paralysis (especially cranial nerves).

### Laboratory diagnosis

The diagnosis of diphtheria is primarily **clinical**, and antitoxin therapy should be initiated without waiting laboratory diagnosis. Microbiological diagnosis serves to confirm the clinical diagnosis. This includes isolation of *C. diphtheriae* and detection of its toxigenicity.

**A. Specimens:** Throat swabs.

#### B. Direct detection in clinical specimens

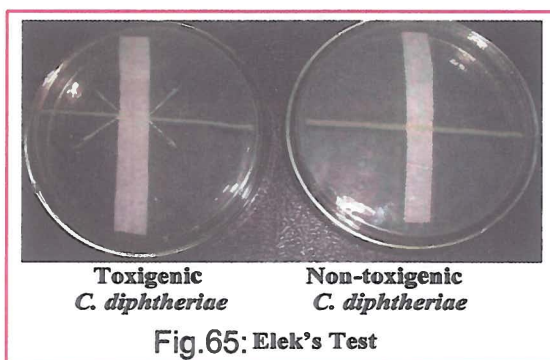
- 1- Gram-stained smears show club-shaped Gram-positive bacilli.
- 2- Methylene blue-stained smears show the characteristic beaded appearance.

#### C. Cultivation

- Culture is done on Loeffler's serum and blood tellurite media.
- A blood agar plate is also used for detection of *S. pyogenes* for differential diagnosis.

#### D. Identification

- *C. diphtheriae* is identified by colony morphology and microscopical examination of stained films.
- Toxigenicity testing of the isolated *C. diphtheriae* strain is done by:
  - 1- Elek's test: The production of diphtheria toxin can be detected by the formation of toxin-antitoxin precipitation bands in the agar. (Fig.65)



- 2- Detection of toxin gene by PCR.
- 3- Detection of toxin from culture by ELISA.

For diagnosis of **carriers**, throat or nasal swabs are subjected to the same procedures including toxigenicity testing.

## Treatment

### A. Diphtheria antitoxin:

- It must be given **immediately**, if diphtheria is clinically suspected.
- Antitoxin will not neutralize toxin that is already fixed to tissues, but will neutralize the circulating (unbound) toxin.
- The antitoxin may be given intramuscularly or intravenously.
- As diphtheria antitoxin is usually produced in horses, precautions to avoid hypersensitivity reactions (anaphylactic shock or serum sickness) should be taken.

**B. Antibiotics:** Penicillin or erythromycin is the drug of choice. Antibiotics inhibit growth of the organism, thus, reducing toxin production. Antibiotic therapy also decreases the incidence of chronic carriers.

**C.** Respiratory support and airway maintenance may be needed.

## Prevention

### Active immunization:

- Diphtheria is a preventable disease by routine active immunization with **diphtheria toxoid**.
- Diphtheria toxoid is prepared by treating the exotoxin with formaldehyde.
- Diphtheria toxoid may be combined with tetanus toxoid (as pediatric DT or adult Td), or with both tetanus toxoid and pertussis vaccine (DPT).
- Vaccine is given by intramuscular injection at 2, 4, 6, and 18 months of age with a booster dose at school entry (6 years). A booster dose of Td is then recommended every 10 years.

### For close contacts:

A booster of diphtheria toxoid with antibiotic chemoprophylaxis are recommended.

## Listeria

### Morphology

Short Gram positive, non spore-forming rods, resembling corynebacteria except in being motile. (Fig.66,67)



Fig.66:  
*Listeria*: similar to *Corynebacteria* in morphology but **MOTILE**

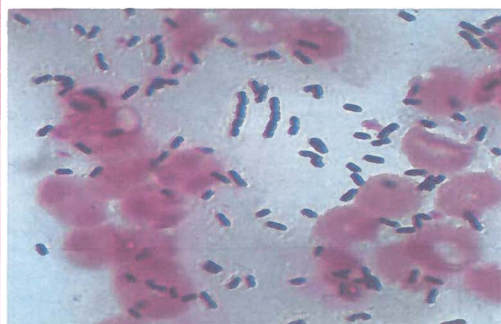


Fig.67: *Listeria* (Gram stain)



## Culture

- *Listeria* grows on blood agar producing  $\beta$ -haemolysis.
- *Listeria* grows well at cold temperature e.g., 4°C (cold enrichment).

## Pathogenesis

- *Listeria* species are found in a diversity of environmental sources including soil, water, food and faeces of humans and animals.
- *Listeria monocytogenes* is the only species that infects humans.
- *Listeria monocytogenes* is transmitted to humans by contact with domestic farm animals or their faeces, by milk or by contaminated vegetables (i.e. food-borne).
- Vertical transmission can also occur transplacentally or during delivery.
- Being a facultative intracellular pathogen, *L. monocytogenes* can survive inside the macrophages due to its ability to produce a membrane-damaging toxin called **listeriolysin-O**. This toxin allows the organism to escape from phagocytic vesicle to enter the cytosole.
- *L. monocytogenes* can move from cell to cell by means of actin rockets, a filament of actin that contracts and propels the bacteria through the membrane of one cell into another. The passage of the organism directly to a neighbouring cell allows avoidance of the immune defensive mechanisms. (Fig.68)

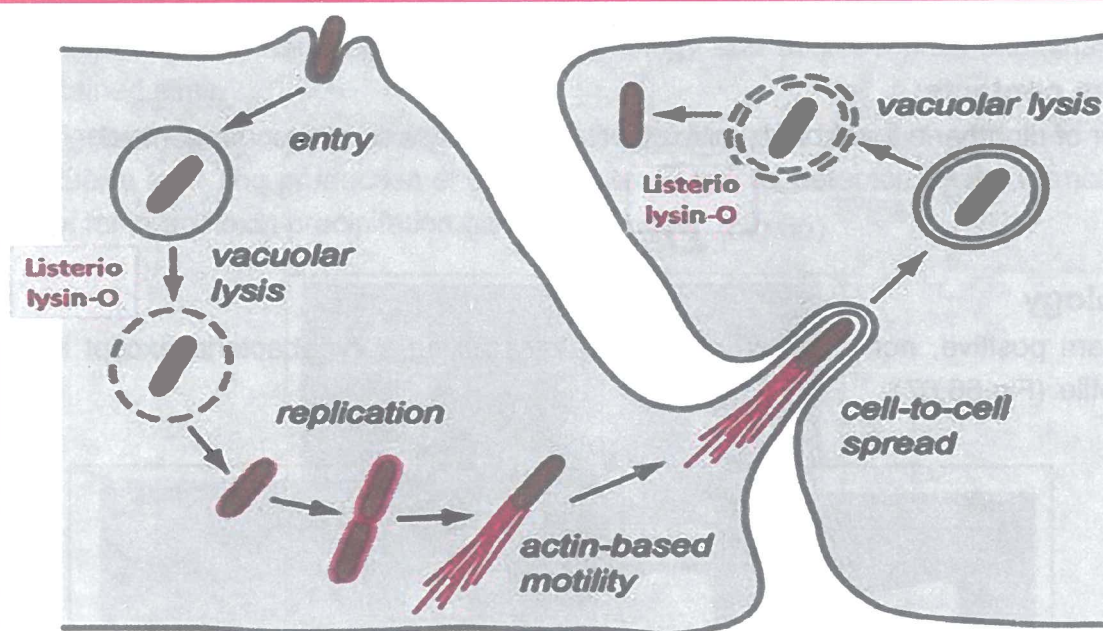


Fig.68: **Pathogenesis of *Listeria***



## Diseases

- 1- Abortion, premature delivery or sepsis during the peripartum period.
- 2- Neonatal meningitis (immunologic immaturity predisposes to serious infection).
- 3- Septicaemia and meningitis in immunocompromised adults.
- 4- Food poisoning:
  - *L. monocytogenes* resists freezing, drying and heat. This enables the organism to survive food processing and cause food poisoning.
  - Outbreaks are usually caused by dairy products (e.g., fresh soft cheese) and under-cooked meat (e.g., chicken and hot dogs).
  - The organism multiplies and invades the intestinal epithelium.
  - Incubation period ranges between 8-48 hours.
  - The disease is characterized by watery diarrhoea, fever and abdominal cramps but little vomiting.

## Treatment

Ampicillin is the drug of choice.

## Gardnerella

- *Gardnerella vaginalis* is a Gram-negative or Gram-variable bacillus.
- *G. vaginalis*, formerly known as *Corynebacterium vaginalis*, is now classified as a separate genus termed *Gardnerella*.
- It is part of the vaginal flora in women, during the reproductive age.
- *Gardnerella vaginalis* is associated with **bacterial vaginosis (BV)**, a non-specific vaginitis that is accompanied by thin vaginal discharge with a bad odour. Women with BV have a higher incidence of preterm labour.
- Laboratory diagnosis is done on vaginal discharge: (Fig.69)
  - Direct Gram-stained smear reveals the presence of clue cells (vaginal epithelial cells covered with *G. vaginalis*), and almost absence of lactobacilli.
  - An increased pH ( $\geq 5$ ) which is due to reduced number of lactobacilli.
  - Whiff test: addition of 10% KOH to the sample gives fishy odour.

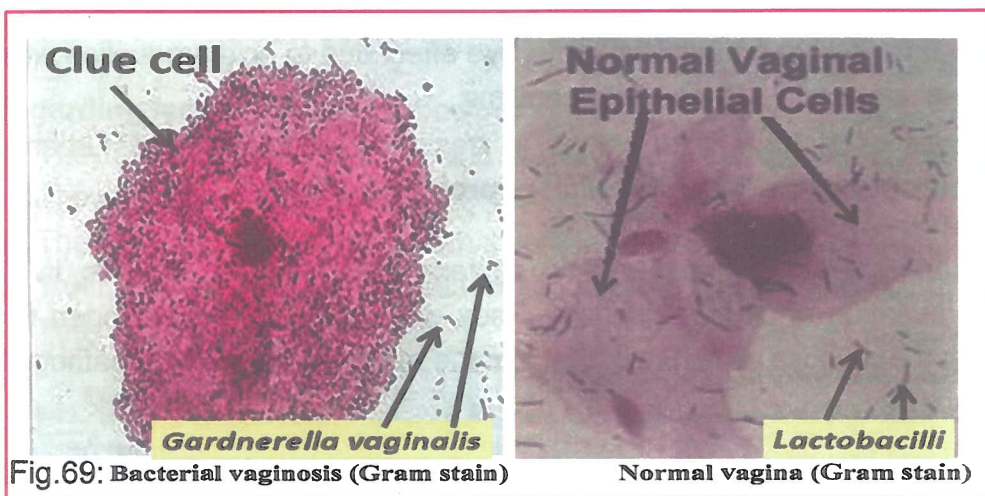


Fig.69: Bacterial vaginosis (Gram stain)

Normal vagina (Gram stain)

## PROPIONIBACTERIUM

- They are anaerobic Gram-positive bacilli, having the typical morphology of diphtheroides.
- Their metabolic products include propionic acid from which the genus name is derived.
- *P. acne* is normally present in human skin and GIT. It is the major contributor to the complex pathogenesis of acne vulgaris.(Fig.70)



Fig.70: Acne vulgaris  
(caused by *Propionibacterium acne*)

## LACTOBACILLUS

- They are anaerobic Gram positive bacilli that produce large quantities of lactic acid by fermentation of carbohydrates (acidogenic). They can survive and grow in acidic media (aciduric) with pH 4-5.
- Lactobacilli are normal commensals of the oral cavity, intestine and vagina.
- Acid production by oral lactobacilli may play a role in the progression of dental caries.
- In the vagina, they have a beneficial protective effect due to acid production which inhibits colonization with pathogenic organisms.
- Living cultures of lactobacilli are used in treatment of intestinal disturbances especially nosocomial diarrhoea in children (probiotic effect).

N.B.: **Probiotics** are live, non-pathogenic bacteria that may be effective in the treatment or prevention of certain diseases. They either exclude the pathogen from binding sites on the mucosa or enhance the immune response against the pathogen.

**MCQs:**

- 1- Diphtheria toxin is produced only by strains of *C. diphtheriae* that are:
  - a- Glucose fermenter
  - b- Club-shaped
  - c- Encapsulated
  - d- Lysogenic
  - e- Beaded
- 2- Active immunization against diphtheria is done using:
  - a. Antitoxin
  - b. Toxoid
  - c. Living attenuated organism
  - d. Killed organism
  - e. Oral vaccine
- 3- A 2 days old baby was admitted to hospital with acute meningitis. Lumbar puncture revealed leucocytosis and Gram-positive short rods. What is the most likely cause of the disease?
  - a- *N. meningitidis*
  - b- *E. coli*
  - c- *Listeria monocytogenes*
  - d- *S. pneumoniae*
  - e- *H. influenzae*
- 4- *Gardnerella vaginalis* is the causative agent of:
  - a- Gonorrhoea
  - b- Bacterial vaginosis
  - c- Ophthalmia neonatorum
  - d- Male urethritis
  - e- Pelvic inflammatory disease
- 5- Regarding lactobacilli, the following statements are correct EXCEPT:
  - a. They are acidogenic and aciduric.
  - b. They have a probiotic effect.
  - c. They constitute part of the normal flora in GIT & vagina.
  - d. Their increase in number is associated with bacterial vaginosis.
  - e. They contribute to dental caries.



## SPORE-FORMING GRAM-POSITIVE BACILLI

### ILOs:

**By the end of this chapter the student should be able to:**

- Classify species of the *Bacillus* genus
- Describe morphology, culture characteristics & growth requirements of *Bacillus anthracis*
- List and outline important virulence factors of *B. anthracis* and *B. cereus*
- Discuss pathogenesis & list diseases of *B. anthracis* and *B. cereus*
- Outline laboratory diagnostic methods of anthrax
- Outline species of *Clostridium* genus associated with human disease
- Describe morphology, culture characteristics & growth requirements of species of *Clostridium* genus associated with human disease
- List and outline important virulence factors of *Clostridium* species associated with human disease
- Discuss pathogenesis & list diseases caused by *Clostridium* species
- Outline laboratory diagnostic methods of diseases caused by *Clostridium* species
- Discuss available measures for prevention & treatment of gas gangrene, tetanus, botulism & pseudomembranous colitis

The Gram-positive rod-shaped bacteria that form endo-spores, have two principal subdivisions:

- The aerobic or facultative anaerobic genus *Bacillus*.
- The anaerobic genus *Clostridium*.

### *Bacillus*

The genus *Bacillus* includes:

- Two important pathogenic species of humans and animals, *Bacillus anthracis* which causes anthrax, and *Bacillus cereus* which causes food poisoning.
- Harmless saprophytes which are found everywhere in nature and are generally called **anthracoids**.

## *Bacillus anthracis*

### Morphology

- Large, rectangular, Gram-positive bacilli arranged in chains. (Fig.71)
- They form oval central non-bulging spores which are stained with modified Ziehl-Neelsen (spore) stain. *B. anthracis* spores may survive in dry soil for years. (Fig.72)

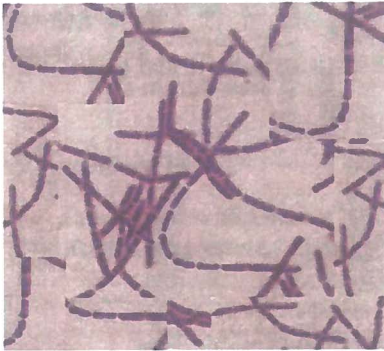


Fig.71: *Bacillus anthracis* (Gram stain)

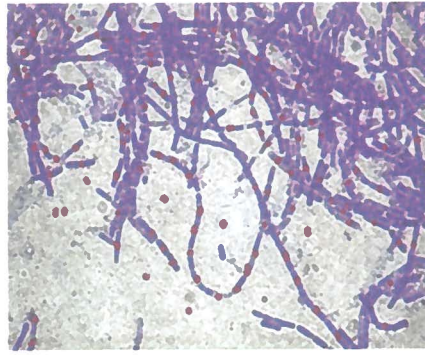


Fig.72: *Bacillus anthracis* (Spore stain)

- They produce a polypeptide capsule which is detected in smears from infected tissues after staining by polychrome methylene blue. The organisms appear as blue rods in a purple/pink-stained capsular material (McFadyean reaction). (Fig.73)



Fig.73: *Bacillus anthracis*  
McFadyean reaction (polychrome MB stain)

### Cultural characters

- *B. anthracis* can be cultivated in simple nutrient media under aerobic or anaerobic conditions.
- *B. anthracis* grows well on blood agar, forming non-haemolytic colonies.



## Anthrax

### Pathogenesis

The pathogenicity of *B. anthracis* depends on two virulence factors:

1. **Capsule:** that protects it from phagocytosis.
2. **Anthrax toxin:** the organism produces three distinct proteins: oedema factor (EF), lethal factor (LF) and protective antigen (PA). (Fig.74)
  - EF complexed with PA is known as oedema toxin, whereas LF complexed with PA is known as lethal toxin.
  - The PA (the B or binding subunit) mediates binding of each toxin to the target tissue. It is called the protective antigen because it has been used in producing a protective vaccine.
  - The EF (the A or active subunit of the oedema toxin) has an adenylate cyclase activity resulting in severe oedema.
  - The LF (the A or active subunit of the lethal toxin) has a protease activity which is responsible for tissue necrosis.

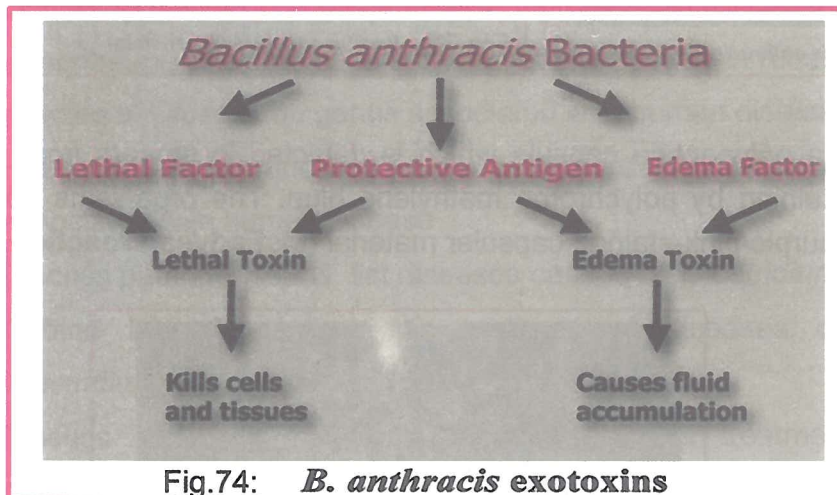


Fig.74: *B. anthracis* exotoxins

### Clinical manifestations

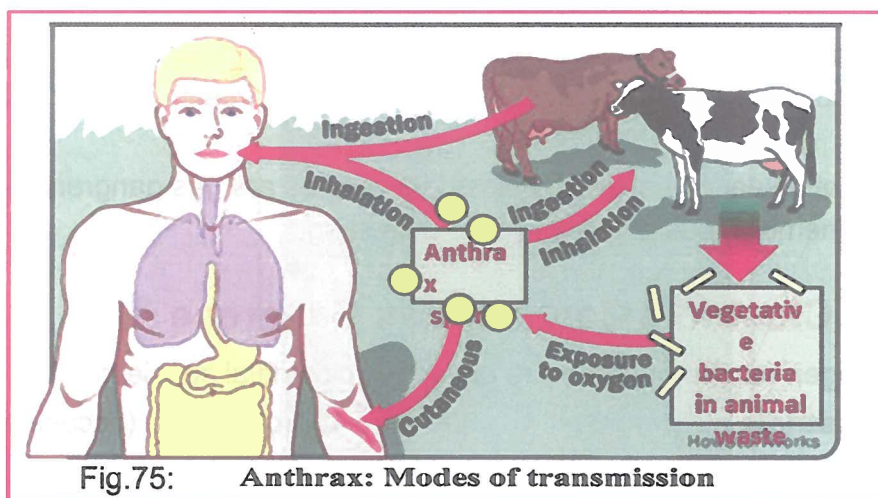
Anthrax is primarily a disease of farm animals. Humans acquire anthrax as a result of contact with infected animals or animal products (**zoonotic disease**). There is no person-to-person transmission of anthrax. The disease takes one of three forms, depending on the route of infection: (Fig.75)

#### 1. Cutaneous anthrax (malignant pustule):

- It occurs from handling infected material. Spores from the soil or from an infected or dead animal enter through a skin cut or abrasion, usually on an exposed area.
- The spores germinate, and vegetative cells multiply locally, producing the anthrax toxin.
- The typical lesion is a painless ulcer with a black eschar surrounded by marked oedema (malignant pustule).
- Untreated cases may develop fatal fulminating septicaemia.



2. **Pulmonary anthrax** is caused by inhalation of spore-laden dust. The possibility of creating aerosols containing anthrax spores has made *B. anthracis* a chosen weapon of bioterrorism.
3. **Intestinal anthrax** occurs rarely through ingestion of infected meat.



### Diagnosis

The clinical diagnosis of anthrax is confirmed by directly visualizing or culturing the anthrax bacilli. Demonstration of capsulated *B. anthracis* from human and animal specimens, even in low numbers, confirms a clinical suspicion of anthrax.

### Treatment

Ciprofloxacin is the drug of choice.

### Prevention

People at high risk can be immunized by a cell-free vaccine containing purified protective antigen (PA).

## *Bacillus cereus*

*Bacillus cereus* causes two types of food poisoning (Table 3).

**Table (3):** Comparison between types of *B. cereus* food poisoning

	The emetic form	The diarrhoeal form
Incubation period	Short (1 to 6 hours)	Long (8 to 16 hours)
Manifestations	Vomiting & abdominal cramps	Diarrhoea & abdominal cramps
Aetiology	Preformed heat-stable enterotoxin (emetic toxin)	Heat-labile enterotoxin (diarrhoeal toxin)
Action of toxin:	Irritation of gastric mucosa	Activation of intestinal adenylate cyclase leading to intestinal fluid secretion.
Associated food	Fried rice (especially from Chinese restaurants)	Meat dishes
Similar to food poisoning caused by	<i>S. aureus</i>	<i>C. perfringens</i>

## *Clostridium*

### Characters of the genus *Clostridium*

1. Large Gram-positive, spore-forming rods.
2. Anaerobic.
3. Most species are motile.
4. The natural habitat of *Clostridium* species is soil or the intestinal tract of animals and humans.

The most important diseases caused by clostridia species are gas gangrene, tetanus, botulism, pseudomembranous colitis and food poisoning.

### Clostridia Causing Gas Gangrene

The primary pathogen can be one or more of various clostridial species:

#### Saccharolytic clostridia

- *C. perfringens* (80%)
- *C. novyi*
- *C. septicum*

#### Proteolytic clostridia (occasional)

- *C. bifermentans*
- *C. histolyticum*
- *C. fallax*

### *Clostridium perfringens*

*Clostridium perfringens* is normally present in the large intestine of man and animals; therefore, it is considered one of the indicators of faecal pollution of water.

### Morphology

Gram positive large rectangular bacilli, with oval sub-terminal spores. (Fig.76)

### Culture

- Anaerobic organism.
- On blood agar, colonies are surrounded by a unique double zone of haemolysis:  $\alpha$ -haemolysis due to production of  $\alpha$ -toxin and  $\beta$ -haemolysis due to production of theta toxin. (Fig.77)

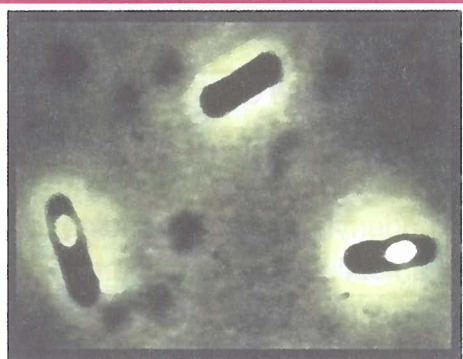


Fig.76: *Clostridium perfringens*  
(oval, subterminal spores)



Fig.77: *Clostridium perfringens* on blood agar  
(double zone of haemolysis)



- On egg yolk agar, *C. perfringens* colonies are surrounded by opaque zones due to production of  $\alpha$ -toxin (**Nagler's reaction**). This effect can be inhibited specifically by antibody against  $\alpha$ -toxin of *C. perfringens*. (Fig.78)

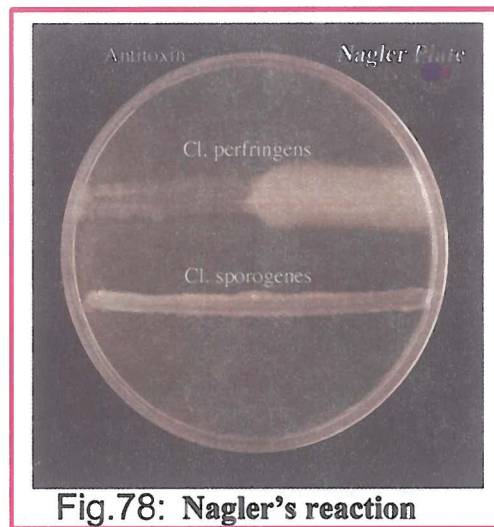


Fig.78: Nagler's reaction

### Virulence factors

*C. perfringens* elaborates at least 12 exotoxins, designated by Greek letters. These exotoxins have haemolytic, cytotoxic and necrotic effects. The most important of these is  $\alpha$ -toxin (lecithinase) which lyses cell membrane lecithins, damaging cell membranes and causing cell death.

### Gas Gangrene

Gas gangrene (or clostridial myonecrosis) is an acute disease with a poor prognosis and often fatal outcome.

### Pathogenesis and clinical findings

- All clostridial wound infections occur in an anaerobic tissue environment caused by an impaired blood supply secondary to trauma, surgery, foreign bodies or malignancy.
- The organism and its spores are found in the soil and in human and animal faeces and, therefore, clostridial wound infections are usually polymicrobial.
- The spores gain access to traumatized tissues by contamination from these sources (faeces or soil).
- Lack of oxygenation allows germination of the spores and growth of clostridia.
- The organisms multiply in the subcutaneous tissues, producing gas and an **anaerobic cellulitis**.
- The organisms invade deeper into the muscle where they produce exotoxins (especially  $\alpha$ -toxin) causing extensive cell death, and enzymes (e.g., hyaluronidase and collagenase) which facilitate the spread of infection.



- Fermentation of tissue carbohydrates yields gas which rapidly accumulates and dissects along tissue planes. The gas bubbles can be felt in the tissues (crepitations).
- Infected muscle is discoloured, oedematous and produces a foul-smelling exudate.
- As the disease process continues, the muscle becomes frankly gangrenous, black and extremely friable. (Fig.79)

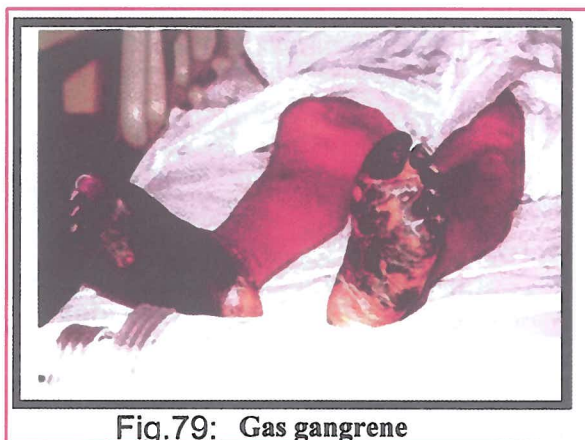


Fig.79: Gas gangrene

- The infection proceeds very rapidly and causes acute pain.
- If the toxins escape from the affected area and enter the bloodstream, it will result in massive haemolysis, renal failure and eventually death.

### Diagnosis

Diagnosis is based on clinical findings. Treatment must be promptly initiated on a clinical basis without waiting for laboratory confirmation because gas gangrene may spread and cause death within hours. Laboratory diagnosis is done as follows:

- A. Specimen** is taken from the depth of the wound and examined rapidly.
- B. Gram-stained smear** shows large Gram positive bacilli. There is typically an absence of PMNs due to the clostridial toxins.
- C. Cultivation** on two blood agar plates, one incubated anaerobically and the second incubated aerobically at 37°C for at least 48 hours.
- D. Identification:** Colonies of *C. perfringens* grow only anaerobically and are further identified by morphology, culture characters, Nagler's reaction and sugar fermentation.

### Treatment and prevention

1. Surgical debridement and removal of foreign materials.
2. Antibiotics: penicillin is the drug of choice.
3. Hyperbaric oxygen chamber: to force oxygen into the wound.
4. Anti-alpha toxin antiserum: may help if given early enough.

## *Clostridium perfringens* Food Poisoning

- *C. perfringens* is a major cause of food poisoning.
- The disease results from ingestion of a large number of organisms in contaminated food, usually meat or meat products. Following ingestion, *C. perfringens* produces an enterotoxin (*in vivo*).
- The incubation period is 8-24 hours after ingestion of contaminated food.
- Symptoms include watery diarrhoea, cramps and abdominal pain.
- The disease lasts only about 24 hours.
- No specific treatment is required. Only fluid therapy is needed to correct the electrolyte imbalance.

## *Clostridium tetani*

*Clostridium tetani* is the causative agent of tetanus. It is found in the intestinal tracts and faeces of various animals and hence its spores are abundant in soil, especially heavily-manured soils.

### Morphology

*C. tetani* is a Gram-positive rod. It forms a bulging terminal spore that gives the organism a characteristic drumstick appearance. (Fig.80)



Fig.80: *Clostridium tetani*

### Culture

- *C. tetani* is a strict anaerobe.
- It grows well on simple and cooked meat media.
- On blood agar, colonies are surrounded by zones of  $\beta$ -haemolysis due to the production of heamolysin.

### Virulence factors

**Tetanospasmin toxin (Neurotoxin):**

- It is of a single antigenic type although it is produced by different strains of *C. tetani*.
- It is among the most toxic substances known.
- The toxin is composed of A and B subunits.



## Tetanus

Tetanus is a highly fatal disease; the mortality rates vary from 40% in adults to 90% in the neonates.

### Pathogenesis

- Tetanus spores are wide-spread in soil and originate from the faeces of domestic animals.
- Most cases of tetanus result from lacerations or small puncture wounds (e.g., nail puncture) contaminated with *C. tetani* spores.
- Germination of spores is favoured by presence of necrotic tissue and poor blood supply in the wound.
- The vegetative cells of *C. tetani* grow locally in the necrotic tissue and elaborate tetanospasmin toxin.
- Tetanospasmin spreads by retrograde transport within the axon and probably haematogenously until it reaches the **central nervous system**.
- The B (binding) subunit mediates binding to gangliosides and penetration of the A (active) subunit.
- The A subunit blocks the release of the inhibitory mediators (glycine and gamma-aminobutyric acid) at spinal synapses, thereby causing severe prolonged muscle spasm (**spastic paralysis**).

### Clinical manifestations

- Incubation period of tetanus varies from 4 days to several weeks, depending on severity of the wound and proximity to the brain.
- Specific clinical features include:
  - Lock jaw (trismus) due to rigid contraction of the jaw muscles. (Fig.81)
  - Arching of the back (opisthotonus) due to spasm of strong extensors of the back. (Fig.82)
  - Exaggerated reflexes to any external stimulus (e.g., noise or light).



Fig.81: Tetanus  
Trismus (spastic paralysis)



Fig.82: Tetanus  
Opisthotonus (spastic paralysis)



- Death is usually the result of paralysis of the chest muscles leading to respiratory failure.
- **Neonatal tetanus** is seen in newborns of **mothers lacking specific immunity**. It occurs as a result of contamination of the umbilical stump with *C. tetani* spores due to lack of aseptic technique during labour.

## Diagnosis

Diagnosis is a **clinical** one. Successful treatment depends on early diagnosis before a lethal amount of toxin becomes fixed to neural tissue. Laboratory diagnosis may be done for confirmation:

- Specimens from the depth of the wound are examined.
- The organism is identified by its morphology and culture characters (see before).

## Treatment

1. **Human tetanus immunoglobulin (HTIG)**: To neutralize the toxin in the blood, tetanus antitoxin is given rapidly. Human rather than horse antitoxin is used to avoid hypersensitivity reactions.
2. Local debridement of the wound is recommended after the patient's spasms are controlled by sedatives and muscle relaxants.
3. Metronidazole or penicillin is usually administered to kill the bacteria and reduce further toxin production.
4. Supportive measures, such as respiratory assistance and intravenous fluids, are often critical to patient survival.

## Prevention and control

- Prophylactic immunization is the only way to control tetanus because the spores of *C. tetani* are so widely disseminated in nature and cannot, therefore, be avoided.
- The elaborated toxin is of a single antigenic type making immunization effective.
- Prophylactic immunization provides the individual with neutralizing antibodies to tetanus toxin in the blood.
- This could be achieved by active immunization by **tetanus toxoid** (formaldehyde-inactivated toxin).
- The toxoid is given to:

### a- Infants:

In the first year of life: The triple vaccine (DPT) is given at the age of 2, 4 and 6 months by intramuscular injection. Booster doses are given at 18 months and upon school entry.

N.B.: Booster doses (Td) are given every 10 years to maintain immunity. More frequent boosters are unnecessary and may cause hypersensitivity reactions.

b- People at high risk, e.g. military personnel.

c- Pregnant females to prevent tetanus neonatorum.

d- Wounded individuals:

Postexposure prophylaxis of wounded individuals (in the form of toxoid with or without HTIG) is performed according to table 4.

**Table (4):** Postexposure prophylaxis against tetanus in wounded individuals

Vaccination status	HTIG	Toxoid	
		Booster dose	Full vaccination
Unvaccinated or unknown	+	-	+
Vaccinated less than 5 years ago	-	-	-
Vaccinated 5-10 years ago	-	+	-
Vaccinated more than 10 years ago	+	+	-

### *Clostridium botulinum*

*Clostridium botulinum* is widely distributed in soil, sediments of lakes and ponds, and decaying vegetation. Hence, the intestinal tracts of mammals, fish and birds may contain the organism.

#### Morphology

*C. botulinum* is a large Gram-positive bacillus that forms oval subterminal spores.

#### Virulence factors

**Botulinum toxins (Neurotoxins):**

- There are several immunologically distinct botulinum toxins. Types A, B and E cause human botulism.
- Type A toxin is the most potent toxin in existence (1 gm would be enough to kill 14,000 people if ingested or 1.25 million people if inhaled!).
- The botulinum toxins are similar in structure to the tetanus toxin (A/B model).

#### Pathogenesis

- Spores, widespread in soil, contaminate vegetables and meat. When these foods are canned without adequate sterilization, spores survive and germinate in the anaerobic environment.
- Toxin is produced within the canned food and ingested **preformed**. The highest-risk foods are vegetables such as green beans and mushrooms, smoked and salted fish, and commercially canned salmon.
- The toxin is absorbed in the intestine and is transported systemically via the bloodstream to reach the peripheral neuromuscular synapses.
- The toxin binds to the neurone and prevents the release of acetylcholine across the synaptic cleft. Thus, it produces paralysis of the motor system (**flaccid paralysis**).



N.B.: Botox is a commercial preparation of exotoxin A used to remove wrinkles of the face. Minute amounts of the toxin are also effective in the treatment of certain spasmodic muscle disorders such as torticollis.

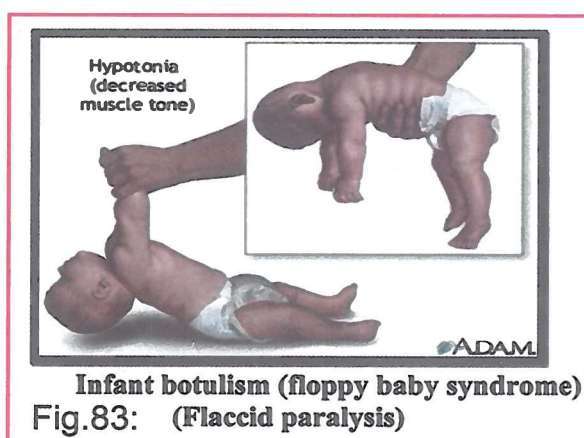
## Clinical forms

### a- Classic botulism

- Manifestations start to appear after an incubation period of 12-36 hours.
- The cranial nerves are affected first (causing blurred vision, inability to swallow and difficulty in speech), followed by a descending, symmetric paralysis of motor nerves. Nausea and vomiting are not usually prominent. No fever is apparent.
- Mortality rate is about 15% and is usually caused by respiratory failure.

### b- Infant botulism

- Ingestion of food supplements containing raw honey contaminated with *C. botulinum* spores has been implicated in transmission of infant botulism.
- In contrast to classic botulism, which is caused by ingestion of preformed toxin, infant botulism results from germination of spores in the gastrointestinal tract where vegetative cells replicate and release the botulinum toxin.
- *C. botulinum* causes the disease in infants between 2 weeks and 6 months of age before the establishment of competing intestinal flora.
- The disease is characterized by constipation, weak sucking ability and generalized weakness (floppy baby syndrome). (Fig.83)
- Almost all cases recover.



### c- Wound botulism

- A rare form of botulism which occurs when a wound becomes contaminated with the organism and toxin is absorbed from that site.
- It may occur among drug addicts (skin popping with heroin).



## Diagnosis

Diagnosis is a **clinical** one but may need laboratory confirmation as follows:

- Demonstration of the toxin in serum, stools and food.
- Rarely, isolation of the organism is done.

## Treatment

- A potent trivalent (A, B, E) antitoxin is available.
- Antitoxin is only effective if it binds to the toxin before the toxin binds the neuromuscular junction (within 12 hours after ingestion).
- Serum sickness may occur because the antitoxin is made in horses.
- There is no reason to give antibiotics except in infant and wound botulism.

## Prevention

- The most important aspect of botulism prevention is proper sterilization of canned food.
- Because the toxin is heat-labile, boiling for 10 minutes or intense heating (cooking) of contaminated food will inactivate the toxin.
- Swollen cans must be discarded.

## *Clostridium difficile*

*C. difficile* is the most common cause of **antibiotic-associated diarrhoea**. The organism is carried in the intestinal tract in approximately 3% of the general population and up to 30% of hospitalized patients. The hands of hospital personnels play an important role in faeco-oral transmission.

## Morphology

*C. difficile* is a spore-forming, Gram-positive bacillus.

## Virulence factors and pathogenesis

- *C. difficile* is a minor component of the normal flora of the large intestine.
- When antimicrobial treatment suppresses more predominant, drug-sensitive members of the normal flora, *C. difficile* multiplies producing exotoxins A and B:
  - Toxin A is mainly an enterotoxin that causes fluid accumulation in the bowel; it also stimulates an inflammatory response.
  - Toxin B is a potent cytotoxin that kills colonic mucosal cells.
- The severity of disease varies widely from mild diarrhoea through varying degrees of inflammation of the large intestine to a fulminant **pseudomembranous colitis**.

(Fig.84)



Fig.84: *C. difficile* Pseudomembranous colitis

## Diagnosis

Pseudomembranous colitis can be differentiated from the transient diarrhoea that occurs as a side effect of many oral antibiotics by:

- Detection of *C. difficile* toxins in stools by ELISA.
- Detection of the toxins genes by PCR.

## Treatment

1. Withdrawal of the causative antibiotic.
2. Treatment with anti-*C. difficile* drugs: Oral vancomycin or metronidazole may be required in an extended course to prevent recurrence (occurs in 15 to 20% of patients).
3. Correction of dehydration and electrolyte imbalance.
4. Antidiarrhoeal agents should **NOT** be taken.
5. Restoration of the patient's colonic flora by faecal enema from a normal individual.

### MCQs:

- 1- The following statements concerning *B. anthracis* are correct **EXCEPT**:
  - a- It is a Gram-positive spore forming bacillus.
  - b- It causes a zoonotic disease.
  - c- It is a typical biological weapon.
  - d- The toxin is its only virulence factor.
  - e- It can cause pneumonia or skin lesions.
- 2- The emetic form of *B. cereus* food poisoning is characterized by the following **EXCEPT**:
  - a- It resembles *S. aureus* food poisoning.
  - b- It has a short incubation period.
  - c- The incriminated food is usually fried rice.
  - d- It is due to heat labile enterotoxin.
  - e- It is manifested by vomiting and abdominal cramps.
- 3- The following is true about *Clostridium perfringens* **EXCEPT**:
  - a- It causes gas gangrene.
  - b- It causes food poisoning.
  - c- It produces an exotoxin that degrades lecithin.
  - d- Endotoxin is a virulence factor of the organism.
  - e- It is one of the indicators of faecal pollution of water.

- 4- Active immunization against tetanus is given to the following **EXCEPT**:
- a- Pregnant females
  - b- Infants in the first year of life
  - c- Military personnel
  - d- Wounded individuals with history of vaccination within 2 years
  - e- Routinely every 10 years
- 5- Symptoms of botulism are due to:
- a- Invasion of the gut epithelium by *C. botulinum*
  - b- Secretion of an enterotoxin
  - c- Ingestion of a neurotoxin
  - d- Endotoxic shock
  - e- Activation of cyclic AMP
- 6- A patient presents with severe colitis associated with an overgrowth of *C. difficile* in the bowel. The most likely cause of this condition is:
- a- Botulinum food poisoning
  - b- A stomach ulcer
  - c- Compromised immune system
  - d- Antibiotic therapy
  - e- Mechanical blockage of the large intestine



## ENTEROBACTERIACEAE

### ILOs:

By the end of this chapter the student should be able to:

- Describe how members of the family *Enterobacteriaceae* are distinguished from other Gram-negative bacteria
- Describe culture characteristics, growth requirements and biochemical reactions of lactose fermenter *Enterobacteriaceae*
- Recognize the means for classification of *E. coli* strains
- Outline important virulence factors of lactose fermenter *Enterobacteriaceae* (*E. coli*, *Klebsiella*)
- Describe and classify species of the *Salmonella* and *Shigella* genera
- Describe culture characteristics, growth requirements and biochemical reactions of lactose non-fermenter Gram negative bacilli
- Outline the antigenic structure of *Salmonella* and *Shigella* species
- Describe diseases caused by *Salmonella* & *Shigella* species
- Outline the laboratory diagnosis & prevention of enteric fever
- Outline the laboratory diagnosis & prevention of bacillary dysentery
- List treatment and prevention measures for enteric fever and bacillary dysentery
- List defining properties for *Proteus*, *Providencia* & *Morganella* species
- List diseases associated with *Proteus*, *Providencia* & *Morganella* species
- List different *Yersinia* species and their pathogenesis
- Outline important virulence factors of *Yersinia pestis* and contrast pneumonic and bubonic plague
- Outline the laboratory diagnosis of plague

The *Enterobacteriaceae* family is a large group of facultative anaerobic, non-spore-forming, Gram-negative bacilli. The natural habitat is the intestinal tract of humans and animals. Some members are widely distributed in the environment in water and soil, and on plants. (Fig.85)

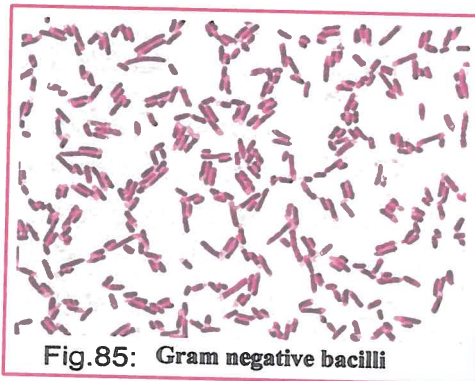


Fig.85: Gram negative bacilli

## General features

Members of *Enterobacteriaceae* share features that help differentiating them from other Gram-negative bacilli:

1. **Facultative anaerobes.**
2. **Oxidase negative.** (Fig.86)

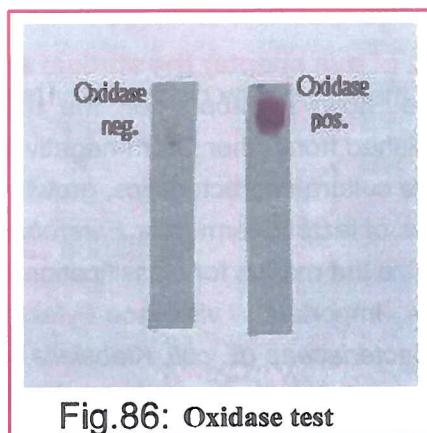


Fig.86: Oxidase test

3. **Ferment glucose.** Fermentation of other sugars is variable; lactose fermentation is an important differential character.
4. **Reduce nitrate to nitrite.**

*Pseudomonas aeruginosa* and *Bacteroides* species which are Gram-negative bacilli normally found in human intestines are considered non-*Enterobacteriaceae*. This is because they do not meet all the criteria of the *Enterobacteriaceae* family, e.g., *P. aeruginosa* is an obligate aerobe, oxidase positive, does not ferment glucose or reduce nitrate, whereas *Bacteroides* is a strict anaerobe.

The family is classified into many genera and species on the basis of biochemical reactions, especially fermentation of carbohydrates, as well as DNA-DNA hybridization. According to their medical importance, they are classified into:

**A. Opportunistic pathogens:** Most *Enterobacteriaceae*, such as *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia* and *Proteus* genera, are commensals and may cause opportunistic infections.

**B. Enteric pathogens:** *Salmonella*, *Shigella*, and *Yersinia* genera, and certain strains of *Escherichia coli* primarily cause intestinal infections and systemic diseases (e.g. typhoid fever caused by *Salmonella Typhi*).

Many strains of these enteric Gram-negative rods are highly antibiotic resistant, because of the production of  $\beta$ -lactamases and other drug-modifying enzymes.

**Extended spectrum  $\beta$ -lactamases (ESBL)** are plasmid-mediated enzymes that are produced by several enteric bacteria, notably *E. coli*, *Klebsiella*, *Enterobacter* and *Proteus*. ESBL render the bacteria resistant to all  $\beta$ -lactam antibiotics (penicillins, cephalosporins and monobactam).

## *Escherichia coli*

*Escherichia coli* is the most predominant facultative anaerobe in the large intestine of man. It has the following medical importance:

1. Being part of the normal bowel flora, *E. coli* provides protection against colonization by harmful microorganisms.
2. Under special circumstances, certain pathogenic strains cause serious intestinal and extraintestinal diseases.
3. In addition to *Enterococcus faecalis* and *C. perfringens*, *E. coli* is used as an indicator of faecal pollution of water. This is because:
  - It is constantly found in human and animal faeces.
  - It is exclusively found in the intestine whereas other members of *Enterobacteriaceae* family are found in the environment as well.

### Morphology

Gram-negative bacilli, usually motile and some are capsulated.

### Cultural characters

*E. coli* grows on MacConkey agar producing rose pink colonies due to lactose fermentation. (Fig.87)

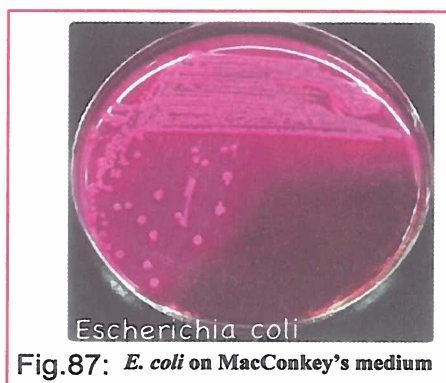
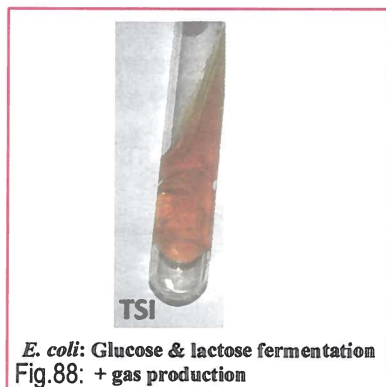


Fig.87: *E. coli* on MacConkey's medium

### Biochemical reactions

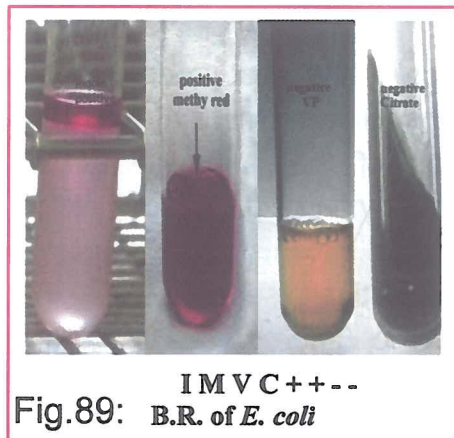
1. *E. coli* ferments glucose, lactose, maltose, mannite and sucrose with production of acid and gas. On TSI, they give acidic (yellow) butt and acidic (yellow) slant with cracking of the agar due to gas production. (Fig.88)



*E. coli*: Glucose & lactose fermentation  
Fig.88: + gas production



2. Indole-positive, methyl red (MR) positive, Voges-Proskauer (VP) negative, and citrate-negative (IMVC ++--). (Fig.89)



### Antigenic structure

- Serological classification of *E. coli* strains is based on:
  - O (somatic) antigen of the cell wall lipopolysaccharide (LPS).
  - H (flagellar) antigen.
  - K (capsular) antigen in capsulated strains.
- Because there are many different O, H, and K antigens, the various combinations result in more than 1000 antigenic types of *E. coli*.
- Specific serotypes are associated with certain diseases e.g., O55 and O111 cause outbreaks of neonatal diarrhoea.

### Virulence factors

#### A. Uropathogenic *E. coli* have:

- Fimbrial adhesins: bind to specific receptors on the urinary tract epithelium.
- Capsular (K) antigens: interfere with phagocytosis, thereby enhancing the organism's ability to cause pyelonephritis.
- Haemolysins: act as membrane-damaging toxins that are linked with kidney damage.

#### B. Diarrhoeagenic *E. coli* have:

1. Pili (colonization factors).
2. Enterotoxins: Two types, heat-labile (LT) and heat-stable (ST), are produced by enterotoxigenic *E. coli* (ETEC).
3. The shiga toxins: are produced by the enterohaemorrhagic *E. coli* (EHEC).

#### C. The LPS causes endotoxic shock when released into the circulation.

## Diseases Caused by *E. coli*

1. **Urinary tract infection (UTI):** The source of *E. coli* that causes UTI is the patient's own colonic flora that colonizes the uro-genital area.
  - a- **Community-acquired UTI:** *E. coli* is the commonest cause and accounts for >80% of infections. The organisms ascend from the periurethral region into urethra (urethritis), bladder (cystitis), ureters, renal pelvis (pyelitis) and renal parenchyma (pyelonephritis).
  - b- **Hospital-acquired UTI:** It is usually associated with urinary catheters and is mostly caused by multi-resistant strains.
2. **Neonatal meningitis:** *E. coli* K1 is a common cause of neonatal meningitis. The source of the organism is the mother's birth canal, where the infection is acquired during birth.
3. **Pneumonia, sepsis, septicaemia, and endotoxic shock** may occur particularly in neonates.
4. **Diarrhoea:** Diarrhoeagenic *E. coli* include several types (Table 5).

## Laboratory diagnosis

### A. Specimens

- Urine, wound swabs, respiratory secretions, blood and CSF (in case of extra-intestinal infections).
- Faeces (in case of diarrhoea).

### B. Direct detection

- 1- Gram-stained smears are useful only in specimens normally devoid of flora, e.g. CSF.
- 2- *E. coli* K1 antigen is detected in CSF in neonatal meningitis by latex agglutination.

### C. Cultivation

- Specimens are plated onto MacConkey agar as well as blood agar and incubated at 37°C.
- Urine should be quantitatively cultured to determine bacteruria (see Vol III).
- Blood samples should be cultivated by the blood culture technique in cases of septicaemia and meningitis. Subcultures are plated on MacConkey agar and blood agar and incubated as above.

Table (5): Types of diarrhoeagenic *E. coli*

Type	Transmission	Pathogenesis	Clinical presentation
1- Enterotoxigenic <i>E. coli</i> (ETEC):	Faeco-oral	Production of enterotoxins (LT and ST)*.	<ul style="list-style-type: none"> <li>- Severe watery diarrhoea in infants and children.</li> <li>- Traveller's diarrhoea in adults.</li> </ul>
2- Enterohaemorrhagic <i>E. coli</i> (EHEC):	Bovine faecal contamination of: <ul style="list-style-type: none"> <li>- Raw or undercooked meat (e.g. hamburger).</li> <li>- Unpasteurized milk.</li> <li>- Raw vegetables.</li> </ul>	Production of verotoxin (shiga-like toxin) most commonly by <i>E. coli</i> serotype O157:H7.	<ul style="list-style-type: none"> <li>- Bloody diarrhea**.</li> <li>- Possible complications**:</li> <li>• Haemorrhagic colitis.</li> <li>• Haemolytic uraemic syndrome (HUS; potentially fatal).</li> </ul>
3- Enteropathogenic <i>E. coli</i> (EPEC):	Faeco-oral	Tight adherence to intestinal mucosa and interference with water absorption.	Watery diarrhoea in infants (infantile diarrhoea).
4- Enteroinvasive <i>E. coli</i> (EIEC):	Faeco-oral	Invasion of colon mucosa, without toxin production.	Bloody diarrhoea (dysentery-like syndrome).
5- Enteragggregative <i>E. coli</i> (Eagg EC):	Faeco-oral	<ul style="list-style-type: none"> <li>- Adherence to intestinal mucosa by aggregative fimbria.</li> <li>- Production of enterotoxin.</li> </ul>	Watery diarrhoea (persistent) in children.

\* LT activates adenylate cyclase resulting in elevation of c-AMP. This induces active secretion of Cl<sup>-</sup> and water by intestinal mucosal cells and inhibits Na<sup>+</sup> reabsorption. The intestinal lumen becomes full of fluids, resulting in watery diarrhoea. LT is similar in structure (A/B subunits) and function to cholera toxin.

ST activates guanylate cyclase resulting in formation of c-GMP, leading to loss of fluids from the intestine.

\*\* The disease is similar to that caused by *Shigella dysenteriae*.

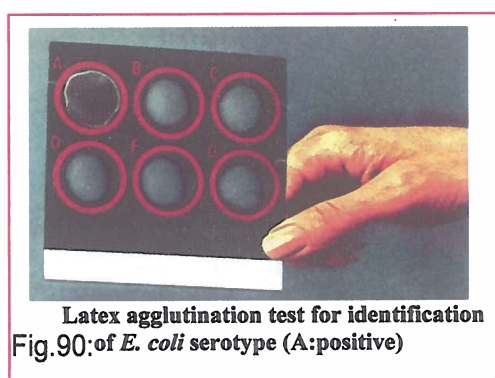


## D. Identification

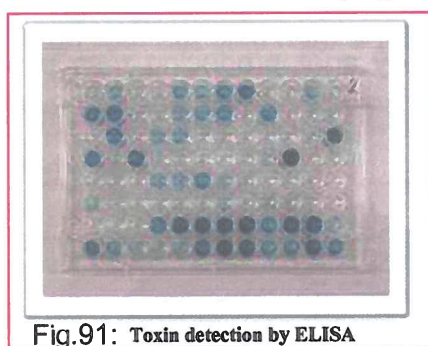
- 1- After 24h incubation, colonies should be examined regarding morphology, Gram stain and oxidase test.
- 2- Colonies showing Gram-negative bacilli and are oxidase negative should be considered *Enterobacteriaceae*.
- 3- *E. coli* produces rose pink colonies on MacConkey agar.
- 4- Colonies should be tested biochemically (see above).

N.B.: Because *E. coli* is part of the normal intestinal flora, strains isolated from faeces of patients with diarrhoea should be further tested to confirm their responsibility for the disease. This may be done as follows:

- **Culture on sorbitol-MacConkey agar:** Unlike other strains of *E. coli*, EHEC does not ferment sorbitol.
- **Slide agglutination:** using specific antisera (EPEC, EHEC). (Fig.90)



- **ELISA:** to test for toxin production (ETEC, EHEC). (Fig.91)



- **DNA probe or PCR:** to detect genes of toxin production (ETEC, EHEC).
- **Tissue culture:** to detect toxin production (ETEC, EHEC), invasiveness (EIEC) or adherence (EPEC, EaggEC).

## Treatment

- Antibiotic therapy of extra-intestinal *E. coli* infections should be guided by *in vitro* susceptibility testing because of the wide spread of resistant strains especially ESBL-producing strains.
- In cases of diarrhoea, treatment depends on correction of dehydration and electrolyte imbalance. Antibiotics may be useful except in cases of EHEC; in such cases antibiotics may increase the risk of developing HUS by increasing shiga toxin released by the dying bacteria.

## *Klebsiella*

Klebsiellae occur in two common habitats:

- 1- The environment: in surface water, sewage, soil and on plants.
- 2- Mucosal surfaces of intestinal and respiratory tracts.

The genus includes *K. pneumoniae*, *K. ozaenae*, *K. rhinoscleromatis* and *K. oxytoca*.

*K. pneumoniae* is the medically most important species.

### Morphology

*Klebsiella* organisms are non-motile capsulated Gram-negative bacilli. (Fig.92)

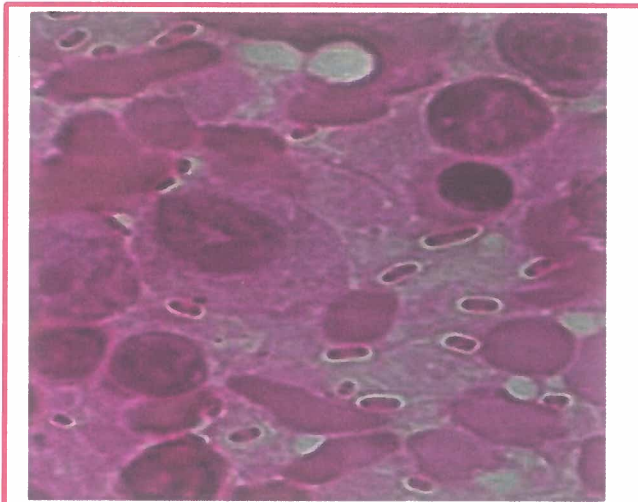


Fig.92: *Klebsiella* in tissue (Gram-stain)

### Culture

*Klebsiella* grows on MacConkey agar producing rose pink colonies due to lactose fermentation. The colonies are usually mucoid due to capsular material. (Fig.93)

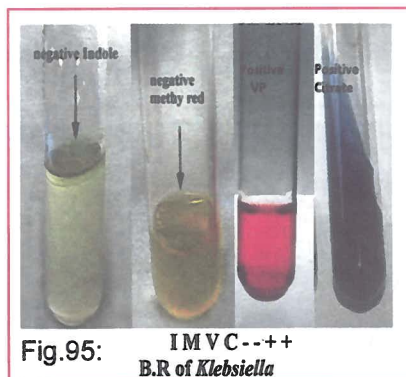
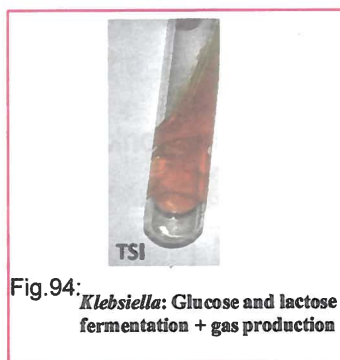


Fig.93: *Klebsiella* (mucoid colonies) on MacConkey's medium

## Biochemical reactions

1- *Klebsiella* ferments glucose, lactose, maltose, mannite and sucrose with production of acid and gas. On TSI, they give acidic (yellow) butt and acidic (yellow) slant with cracking of the agar due to gas production. (Fig.94)

2- IMVC of *K. pneumoniae* is - - + +. (Fig.95)



## Pathogenesis

- The capsule is the most important virulence factor.
- *Klebsiella* infections are either community- or hospital-acquired.
- *K. pneumoniae* and much less frequently *K. oxytoca* may cause:
  1. Urinary tract infection: It is the most common infection.
  2. Pneumonia: *Klebsiella* is carried in the respiratory tract of about 10% of healthy people who are prone to pneumonia if host defence is lowered.
  3. Wound and bloodstream infections.
  4. Neonatal sepsis: Septicaemia or meningitis.
- *Klebsiella ozaenae* is associated with atrophic rhinitis (ozena), a fetid, progressive atrophy of nasal mucosa.
- *Klebsiella rhinoscleromatis* is associated with rhinoscleroma, a destructive granuloma of the nose and pharynx.

## Treatment

Emerging antimicrobial resistance among *Klebsiella* spp. is increasing. Resistance is mediated by  $\beta$ -lactamases (especially ESBL). Therefore, routine *in vitro* susceptibility testing is required.

## *Citrobacter*, *Enterobacter* and *Serratia*

Organisms of these genera are motile Gram-negative bacilli, typically found in soil, water and occasionally in the human respiratory and intestinal tracts and animal intestine. They cause opportunistic infections in humans especially pneumonia and urinary tract infection.

***Citrobacter*:** It is similar to *E. coli* except in being citrate-positive.

***Enterobacter*:** It is similar to *Klebsiella* except in being motile.

***Serratia*:** Some strains produce red non-diffusible endo-pigment and are used for testing efficiency of bacterial filters.



## Salmonella

Salmonellae are widespread in nature. They are commonly found in the intestinal tract of mammals, birds (particularly poultry) and reptiles.

### Morphology

Salmonellae are Gram-negative bacilli and almost all are motile.

### Culture

- *Salmonella* grows as pale lactose-nonfermenting colonies on MacConkey and desoxycholate citrate agar (DCA) media. (Fig.96,97)



Fig.96: *Salmonella* on MacConkey's medium



Fig.97: *Salmonella* on DCA medium

- Salmonella-Shigella (SS) agar is superior to MacConkey and DCA media in detecting  $H_2S$ -producing *Salmonella* species that give black colonies. (Fig.98)



Fig.98: *Salmonella* Typhi on Salmonella Shigella (S-S) agar

- Selenite and tetrathionate broth (enrichment media) are used to isolate *Salmonella* from stools. (Fig.99)

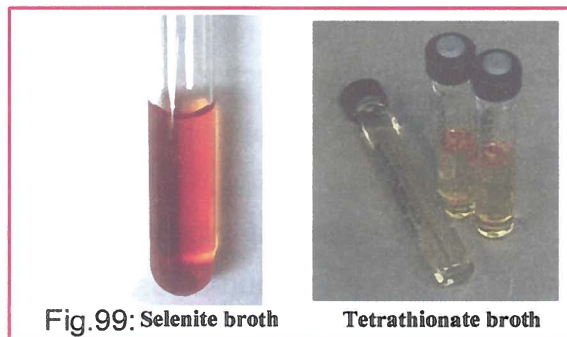


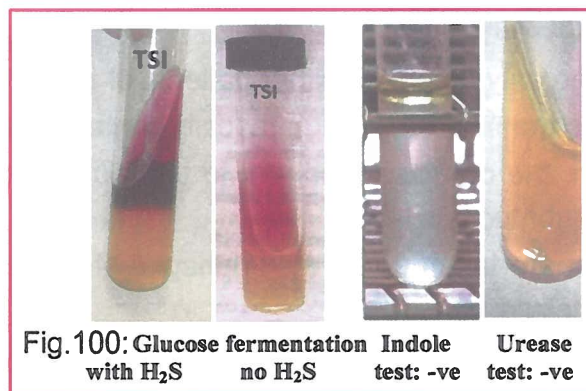
Fig.99: Selenite broth

Tetrathionate broth

## Biochemical reactions

1. Fermentation of glucose, maltose and mannite with production of acid and gas.  
*Salmonella* Typhi produces acid only.
2. Urease-negative and indole-negative.
3. Most species produce  $H_2S$ .

N.B.: On TSI, salmonellae give acidic (yellow) butt and alkaline (red) slant with cracking of the agar (except in case of *S. Typhi*). Black colour develops in case of  $H_2S$  production. (Fig.100)



## Antigenic structure

The antigens used to define groups and types of salmonellae include:

1. The O (somatic) antigens: which divide *Salmonella* into O serogroups. The O antigen may be shared between different groups. Therefore, cross reactions may occur between these groups during serologic testing.
2. The H (flagellar) antigens: which divide the serogroups into serotypes.
3. The Vi (capsular polysaccharide) antigen: which may be present in some strains of *S. Typhi*.

## Classification

- According to DNA hybridization studies: the *Salmonella* genus is classified into two species. The most important species is *Salmonella enterica*, which is further classified into six subspecies. The most important subspecies is the subsp. *enterica* whose strains are isolated from humans and warm-blooded animals.
- According to antigenic structure: the genus *Salmonella* is classified into serogroups and serotypes (~ 2400 serotypes).
- Clinically: *Salmonella* species are categorized into:
  - Typhoidal species: *Salmonella* Typhi (causing typhoid fever), *S. Paratyphi* A, *S. Paratyphi* B and *S. Paratyphi* C (causing paratyphoid fever).
  - Nontyphoidal species: *S. Enteritidis* and *S. Typhimurium* that cause enterocolitis and *S. Choleraesuis* that causes septicaemia and metastatic infections.

## Typhoid and Paratyphoid Fever (Enteric Fever)

Enteric fever is a generalized infection of the reticuloendothelial system and intestinal lymphoid tissue accompanied by bacteraemia and sustained fever. Typhoid fever is more common and tends to be more severe than paratyphoid fever.

### Pathogenesis (Fig.101)

- Humans are the only reservoir, either patients or healthy carriers.
- The infection is transmitted through faecally contaminated food and water.
- After ingestion, the organisms adhere to the mucosa of the small intestine, then invade to the submucosal layer.
- The organisms are taken up by macrophages of the Peyer's patches where they replicate intracellularly.
- They are transported in the macrophages to the mesenteric lymph nodes and via the thoracic duct to the bloodstream (transient 1<sup>st</sup> bacteraemia).
- The organisms reach organs where cells of the reticuloendothelial system are concentrated (i.e. the spleen, bone marrow, liver and Peyer's patches).
- The organisms multiply in these organs and then reinvade the blood causing a 2<sup>nd</sup> heavier bacteraemia (onset of fever).
- From the blood, the organisms can reach other organs (e.g. kidney and gall bladder).
- From the gall bladder, salmonellae enter the intestine for a second time in much larger numbers causing a strong inflammatory response.
- The organism is excreted with faeces (second week of the disease). In about 25% of cases, the organism is excreted in the urine.
- The incubation period is usually 10-14 days.
- Symptoms include fever, headache, abdominal pain, and constipation rather than diarrhoea.
- Complications include relapse, perforation of the bowel and haemorrhage from the bowel ulceration.
- A small percentage of patients become chronic carriers. They carry the organism in the gall bladder and intermittently excrete it in the stools.



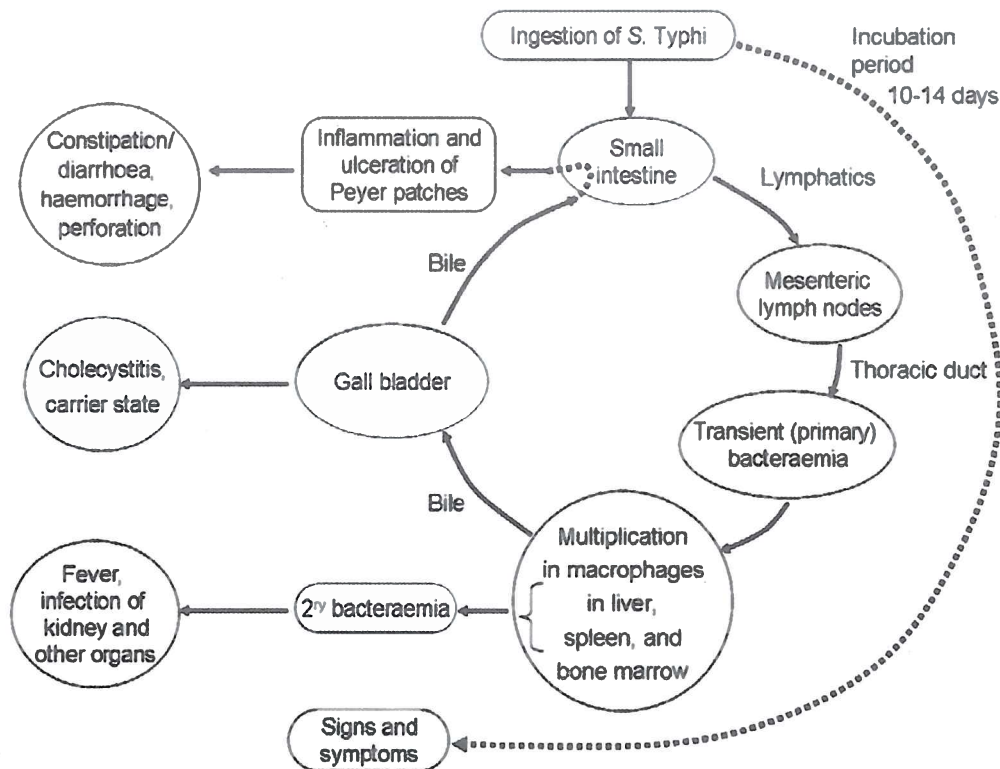


Fig.101: Pathogenesis of typhoid fever.

### Laboratory diagnosis of enteric fever

Definitive diagnosis is only accomplished by isolation of salmonellae from clinical specimens.

#### A. Specimens

- 1- **Blood:** Blood culture is the procedure most likely to reveal the organism during the first two weeks of illness.
- 2- **Faeces:** The organism is most readily isolated from stools starting from the second week of illness.
- 3- **Urine:** Intermittent excretion of the organism in urine may occur after the second week of illness.
- 4- **Bone marrow:** Although highly sensitive, it is rarely indicated because it requires an invasive technique.

**B. Direct detection** by microscopy is useless.

#### C. Cultivation

- 1- **Blood or bone marrow:** Samples should be cultivated by the blood culture technique. Subcultures are plated on MacConkey agar.
- 2- **Stools:** Faecal specimens should be inoculated directly on both:
  - Differential selective solid media such as MacConkey, DCA or Salmonella-Shigella (SS) agar.
  - Enrichment (tetrathionate or selenite) broth. Subcultures on solid media are done after overnight incubation.

### D. Identification

- 1- After 24h incubation, colonies should be examined regarding morphology, Gram stain and oxidase test.
- 2- Colonies showing Gram-negative bacilli and are oxidase negative are considered *Enterobacteriaceae*.
- 3- *Salmonella* produces pale (lactose-nonfermenting) colonies on MacConkey agar and DCA, and black colonies on SS agar.
- 4- *Salmonella* is identified by testing colonies for biochemical reactions (see before).
- 5- Confirmation is done by slide agglutination using O serogroup antibodies. (Fig.102)

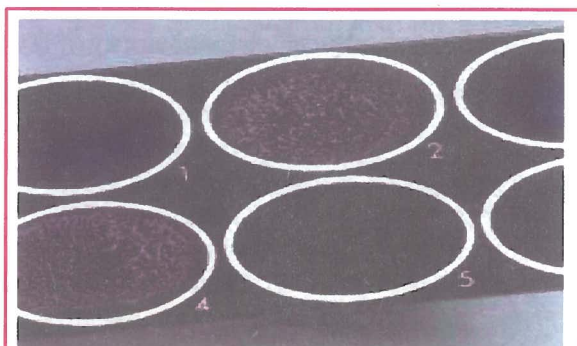


Fig.102: Slide agglutination test for identification of *Salmonella* serogroups

### E. Serodiagnosis of enteric fever by Widal test (Fig.103)

If properly performed and carefully interpreted, Widal test would be diagnostic.

- Widal test is a tube agglutination test that measures agglutinating antibodies to the O and H antigens of *Salmonella* Typhi and Paratyphi A and B.
- Antibodies can be detected at the beginning of the **second week** onwards.
- It is done by mixing serial (twofold) dilutions of the patient's serum with O and H antigens from representative salmonellae.
- The antibody titre is the highest serum dilution showing agglutination.
- The results are reported by giving the titre for both O and H antibodies.
- **Rising of antibody titre** is detected by testing 2 serum samples obtained at an interval of 7-10 days.
- O antibody appears early and its presence signifies active infection. It disappears faster than H antibody, the presence of which determines the type of infecting organism.

### Interpretation of Widal Test:

1. High titre of O and H antibodies ( $\geq 1:160$ ) or rising titre (fourfold or greater) suggest active infection.
2. High titre of H antibody alone ( $\geq 1:160$ ) indicates past immunization or past infection. (Fig.103)

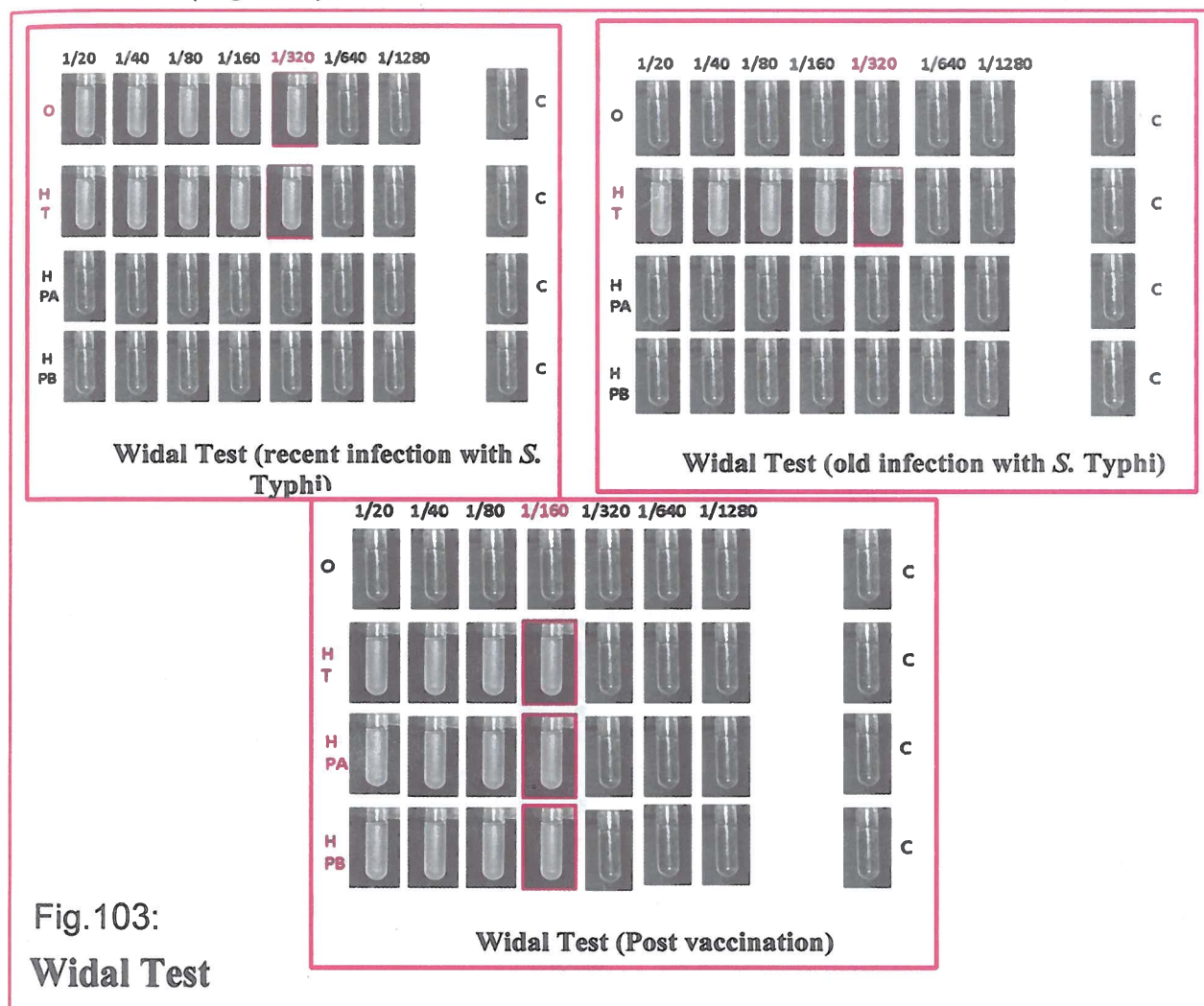


Fig.103:  
Widal Test

### False positive results:

1. The presence of **cross-reacting antibodies**: e.g. infection with other related members of *Enterobacteriaceae* or autoimmune diseases.
2. **Endemicity** of the disease: Healthy people have antibody titre due to subclinical infection. In Egypt, titres up to 1:80 are considered insignificant.
3. **Vaccination**: Vaccinated people have both O and H antibodies if recently immunized and H antibody alone in cases of past vaccination.

### False negative results:

1. Performance of the test during the first week of illness (before appearance of antibodies).
2. Early antibiotic treatment.



## Treatment

- The drug of choice is either fluoroquinolones (e.g. ciprofloxacin) or 3<sup>rd</sup> generation cephalosporins (e.g. ceftriaxone).
- Ampicillin or ciprofloxacin should be used in chronic carriers of *S. Typhi*.

## Prevention

### A. Hygienic measures

- 1- Proper sewage treatment and chlorinated water supply.
- 2- Hand wash prior to food handling.
- 3- Food handlers are examined to diagnose carriers, who should be treated or excluded from handling food.

### B. Immunological measures

- 1- **Oral typhoid vaccine:** A live avirulent mutant strain of *S. Typhi*.
- 2- **Vi capsular polysaccharide vaccine** of *S. Typhi*. It is given intramuscularly.
- 3- **TAB vaccine:** A heat killed vaccine containing *S. Typhi*, *S. Paratyphi A* and *S. Paratyphi B*. The vaccine is given subcutaneously.

## Salmonella Food Poisoning (Gastroenteritis or Enterocolitis)

- It is a worldwide infection caused by *S. Enteritidis* and *S. Typhimurium*.
- Disease transmission is usually linked to food of animal and poultry origins (particularly raw eggs). Water or food contaminated with rat excreta can also transmit the infection.
- The organism invades and replicates in the epithelial cells of small and large intestines (not in macrophages) leading to inflammatory lesions and diarrhoea. There is no toxin production.
- The incubation period is 8-48 hours.
- Manifestations include fever, nausea, vomiting, severe diarrhoea, and abdominal cramps. (Fig.104)

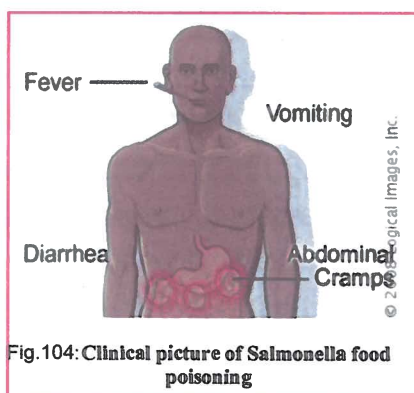


Fig.104: Clinical picture of Salmonella food poisoning

- The condition is usually self-limited, lasting only for few days.
- Diagnosis is made by isolation of the organism from stools.
- Treatment depends on correction of dehydration and electrolyte imbalance. Antibiotic therapy is not needed except in immunocompromised individuals.
- The disease is prevented by avoiding contamination of food and water by rodent excreta, and proper cooking of poultry and eggs.

## Septicaemia

- The condition is commonly caused by *S. Choleraesuis*.
- It usually occurs in immunocompromised individuals.
- After ingestion of salmonellae, the organisms invade the intestinal mucosa and invade the bloodstream early without intestinal lesions. Bacteraemia results in metastatic infections such as osteomyelitis, arthritis, pneumonia and meningitis.
- Blood culture is usually positive particularly during the high fever.

## Shigella

*Shigella* species cause bacillary dysentery in man.

### Morphology

Shigellae are non-motile Gram-negative bacilli.

### Culture

Shigellae grow as pale lactose-nonfermenting colonies on MacConkey and DCA media. (Fig.105,106)

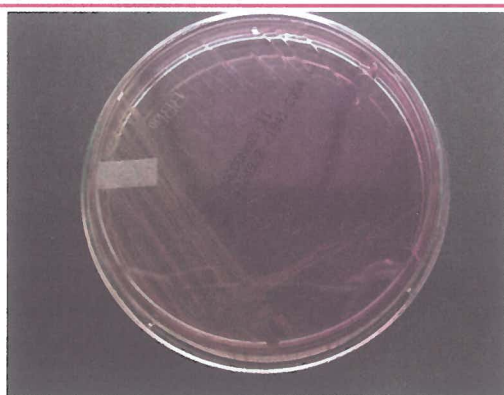


Fig.105: *Shigella* on MacConkey's medium

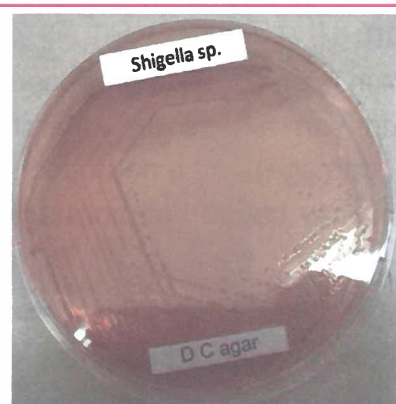
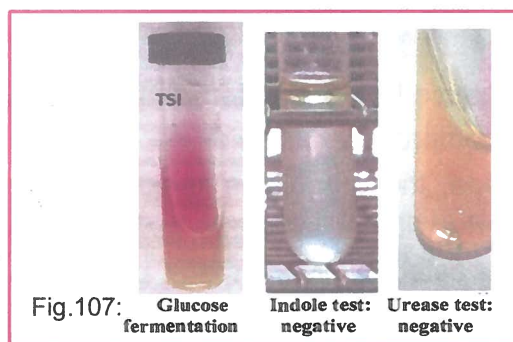


Fig.106: *Shigella* on DCA medium

### Biochemical reactions

- Shigellae ferment glucose, with production of acid only.
- They are lactose-nonfermenters.
- All shigellae are urease and  $H_2S$  negative.
- On TSI, they give acidic (yellow) butt and alkaline (red) slant with no gas and no  $H_2S$  production. (Fig.107)



### Antigenic structure

Shigellae have O antigens which divide the genus into four serogroups (species).

- Group A: *S. dysenteriae* (13 serotypes).
- Group B: *S. flexneri* (8 serotypes).
- Group C: *S. boydii* (18 serotypes).
- Group D: *S. sonnei* (1 serotype).

### Virulence factors

**A. Invasiveness:** The essential pathogenic process of bacillary dysentery is invasion of mucosal epithelium of the terminal ileum and large intestine where the organism is able to grow. The organism does not invade into the blood.

**B. Shiga toxin:**

- Shiga toxin is an exotoxin produced by *S. dysenteriae* type 1.
- The toxin can act as an enterotoxin, a cytotoxin (that may contribute to mucosal damage) and a neurotoxin (that may cause meningismus and coma).
- The toxin may cause haemorrhagic colitis and haemolytic uraemic syndrome (HUS).

### Pathogenesis

- Man is the only reservoir for shigellosis.
- Infections with *Shigella* are often acquired by food or water contaminated with human faeces.
- Ingestion of few organisms (~ 100 organisms) is able to cause disease, so person-to-person transmission can occur.
- The incubation period is 1-4 days.
- The disease is characterized by fever, abdominal cramps, tenesmus and diarrhoea with blood, pus and mucus in stools.
- Severity depends on age of patients and the strain; *S. dysenteriae* type 1 causes the severest form of the disease due to shiga toxin production.

### Laboratory diagnosis

**A. Specimens:** Fresh stools.

**B. Direct detection:** Direct microscopy is done to differentiate bacillary from amoebic dysentery. In bacillary dysentery, large number of PMNLs and some erythrocytes are seen under the microscope.



**C. Cultivation:** Faecal specimens should be initially inoculated onto both:

- Solid media such as MacConkey, DCA or SS agar.
- Enrichment broth (selenite broth). Subcultures on solid media are done after overnight incubation.

**D. Identification**

- 1- After 24h incubation, colonies should be examined regarding morphology, motility, Gram stain and oxidase test.
- 2- Colonies showing Gram-negative bacilli and are oxidase negative are considered *Enterobacteriaceae*.
- 3- *Shigella* produces pale (lactose-nonfermenting) colonies on MacConkey agar and DCA.
- 4- *Shigella* is identified by testing the colonies for biochemical reactions (see above).
- 5- Confirmation is done by slide agglutination test using O serogroup antibodies. (Fig.108)

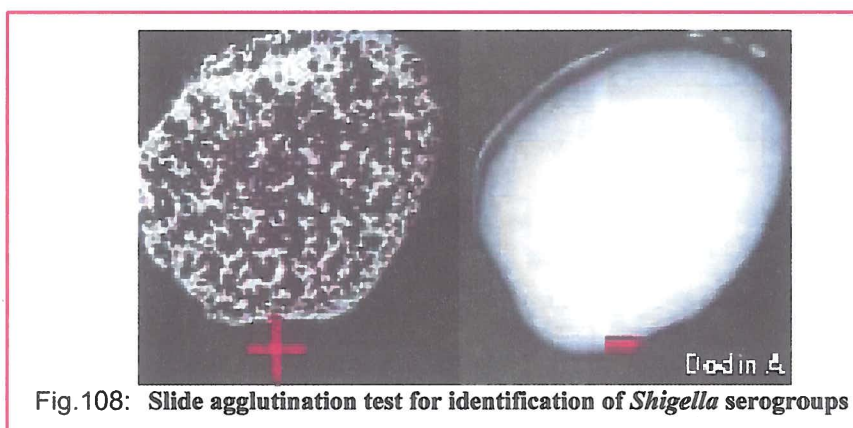


Fig.108: Slide agglutination test for identification of *Shigella* serogroups

N.B.: *Shigella* is distinguished from *Salmonella* by 3 criteria:

1. Non-motile.
2. No H<sub>2</sub>S production.
3. No gas production from glucose fermentation.

**Treatment**

- Fluid and electrolyte replacement for mild cases.
- Antibiotics are recommended for treatment of severe *Shigella* infection (fluoroquinolones e.g. ciprofloxacin, and trimethoprim/ sulfamethoxazole).

**Prevention and control**

Public health measures are recommended for control of shigellosis.

## *Proteus, Providencia and Morganella*

- These organisms occur in the environment as well as in human and animal intestines. Important species include *Pr. mirabilis*, *Pr. vulgaris*, *Providencia rettgeri* and *M. morganii*.
- These 3 genera have the following common diagnostic features:
  - 1- Motile pleomorphic Gram-negative bacilli.
  - 2- Lactose-nonfermenter (LNF).
  - 3- Urease-positive.
  - 4- Phenylalanine deaminase-positive. (This distinguishes them from other members of *Enterobacteriaceae*).
- *Proteus* can be differentiated from:
  - *Providencia* and *Morganella* by its ability to swarm on nutrient agar. (Fig.109)
  - *Salmonella* in stools (both are LNF and H<sub>2</sub>S positive) by urease production. (Fig.110)



Fig.109: *Proteus* on nutrient agar (showing swarming growth)

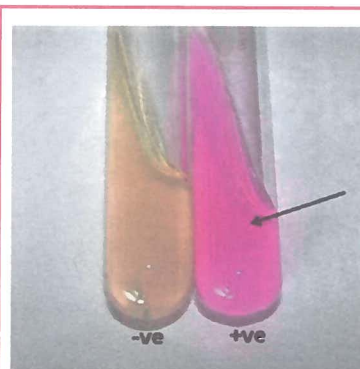


Fig.110: Urease test

### Diseases

1. Urinary tract infection especially caused by *Proteus*. Urease hydrolyses the urea in urine to form ammonia which raises the pH encouraging the formation of stones (calculi).
2. Wound infections and abscess formation.
3. Respiratory infections: Otitis media and pneumonia.
4. Septicaemia and meningitis.

**Treatment:** Antibiotic susceptibility testing should be performed because of the high frequency of resistance to antibiotics.

## *Yersinia*

The genus *Yersinia* includes 3 important human pathogenic species: *Y. pestis*, the aetiologic agent of plague (black death), and the enteropathogenic species, *Y. pseudotuberculosis* and *Y. enterocolitica*.

### *Yersinia pestis*

*Y. pestis* is one of the most virulent bacteria known; 1-10 organisms are capable of causing disease.

Plague is a zoonotic disease primarily affecting rodents which act as reservoirs.

#### Morphology

*Y. pestis* are non-motile Gram-negative coccobacilli that have characteristic bipolar staining, producing a "closed safety pin" appearance. (Fig.111)

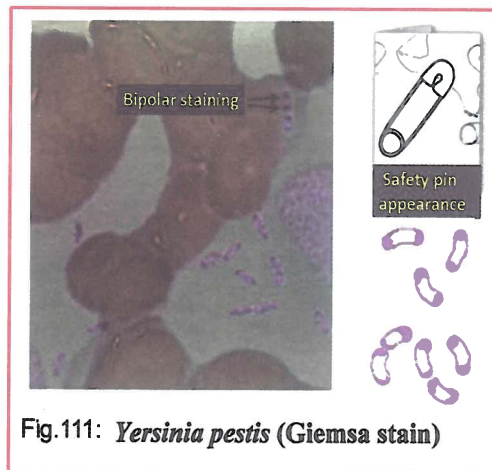


Fig.111: *Yersinia pestis* (Giemsa stain)

#### Cultural characteristics

*Y. pestis* can be cultured on blood agar and MacConkey agar forming small pinpoint (lactose-nonfermenting) colonies after 24 h incubation at 25°C.

#### Virulence factors

1. **Fraction 1 (F1) antigen:** It is an antiphagocytic capsular-like envelope of polysaccharide-protein complex.
2. **Endotoxin:** responsible for endotoxic shock.
3. **Yersinia outer proteins (Yop):** They inhibit phagocytosis and cytokine production.



## Pathogenesis

Transmission of plague between rodents is accomplished by the rat flea (*Xenopsylla cheopis*). The flea acquires *Y. pestis* from an infected blood meal from a bacteraemic rodent. Man can be accidentally infected by one of the following ways: (Fig.112)

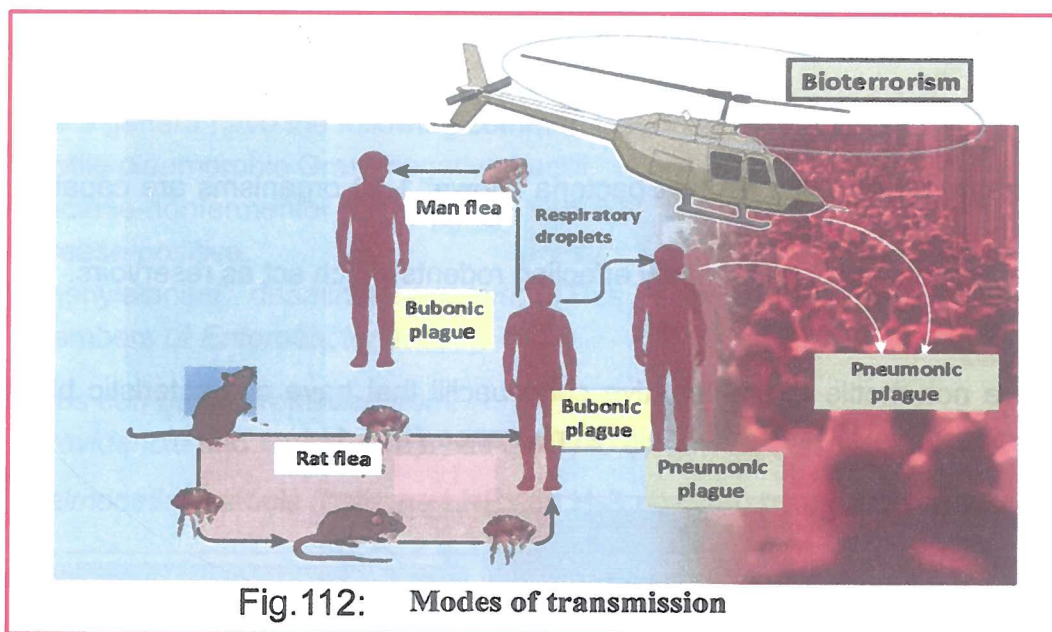


Fig.112: Modes of transmission

1. Flea-borne, from infected rodents through the bite of the flea. This results in **bubonic plague** which is characterized by pain and swelling of the lymph nodes draining the site of the flea bite.(Fig.113)



Fig.113: Bubonic plague

2. Inhalation of respiratory droplets from humans or animals with pneumonic plague, or from aerosol created during handling yersinia culture in the laboratory.

**Pneumonic plague** is characterized by fever, chills, cough and difficulty in breathing; shock and death occur eventually if not treated early.

In either cases, **septicaemic plague** may occur as a result of spread of the organism to the bloodstream. Through haematogenous spread, the organism may reach other organs.

N.B.: In **bioterrorism**, the organism may be delivered by aerosol to cause pneumonic plague, or by using infected fleas to cause bubonic plague.

## Laboratory diagnosis

**A. Specimens:** Lymph node aspirates, sputum or blood.

**B. Direct detection**

- 1- Gram or Giemsa stain reveal the characteristic bipolar staining of coccobacilli.
- 2- Fluorescent-antibody (FA) test to detect F1 antigen.

**C. Cultivation and Identification**

Although hazardous, culture may be done on blood and MacConkey's agar, and the colonies are identified by FA test.

## Treatment

Streptomycin is the drug of choice.

## Prevention

- Anti-rat and anti-flea measures.
- Avoidance of sick or dead animals.

## *Yersinia enterocolitica* and *Y. pseudotuberculosis*

- *Y. enterocolitica* and *Y. pseudotuberculosis* are rare causes of enterocolitis.
- They are transmitted to humans by food contaminated with excreta of domestic animals.
- Refrigerated foods are potential vehicles because *Y. enterocolitica* can grow at refrigeration temperatures.
- Disease (yersiniosis) can range from mild diarrhoea to what appears to be acute appendicitis.

### MCQs:

- 1- The following bacteria belong to *Enterobacteriaceae* family EXCEPT:
  - a- *Escherichia*
  - b- *Shigella*
  - c- *Yersinia*
  - d- *Salmonella*
  - e- *Pseudomonas*
- 2- *E. coli* that produces shiga-like toxin is:
  - a- Enterotoxigenic *E. coli*
  - b- Enterohaemorrhagic *E. coli*
  - c- Enteroinvasive *E. coli*
  - d- Enteroaggregative *E. coli*
  - e- Enteropathogenic *E. coli*

- 3- *Klebsiella* species are characterized by all the following **EXCEPT**:
- a- Resistant strains are important agents of nosocomial infections.
  - b- They may cause urinary tract infection and neonatal sepsis.
  - c- They have a thick polysaccharide capsule.
  - d- Some species may cause rhinoscleroma.
  - e- They are indole positive.
- 4- The following statements describing *Salmonella* species are all correct **EXCEPT**:
- a- They are gram-negative bacilli.
  - b- They grow as pink colonies on MacConkey and DCA media.
  - c- They are urease-negative.
  - d- They cause food poisoning.
  - e- They cause enteric fever.
- 5- Concerning the Widal test:
- a- It is a toxin-antitoxin neutralization test.
  - b- It is positive on the third day of fever.
  - c- Early treatment leads to high titres.
  - d- Titre >1:160 against O and H antigens indicates active infection.
  - e- Titres up to 1:160 are considered normal.
- 6- TAB vaccine is used for protection against:
- a- Meningitis
  - b- Enteric fever
  - c- Tuberculosis
  - d- Diphtheria
  - e- Tetanus
- 7- Regarding *Shigella dysenteriae* all the following statements are correct **EXCEPT**:
- a- It is a member of *Enterobacteriaceae*.
  - b- It produces a toxin that may result in renal failure.
  - c- It grows on MacConkey's medium as colourless colonies.
  - d- Serological identification depends on O and H antigens.
  - e- It causes bloody diarrhoea.
- 8- *Proteus* can be differentiated from *Salmonella* by being:
- a- Gram-negative bacilli
  - b- Non-lactose fermenter
  - c- Urease-positive
  - d- H<sub>2</sub>S-positive
  - e- Motile



## VIBRIO

### ILOs:

By the end of this chapter the student should be able to:

- List defining properties of *Vibrio* species
- Describe culture characters and biochemical reactions of *Vibrio cholerae*
- Recognize the means for classification of *V. cholerae* strains
- Describe the virulence factor and pathogenesis of *V. cholerae*
- Outline diagnostic measures for cholera
- List treatment and discuss prevention of cholera
- Describe the pathogenesis and treatment of diseases caused by other *Vibrio* species
- Recognize the difference between *Aeromonas* and *Vibrio* species, and list diseases caused by *Aeromonas hydrophila*

Vibrios are one of the most common organisms in both sea and freshwater habitats and in association with aquatic animals.

*Vibrio cholerae* is the most clinically important species. Other important species are *V. parahaemolyticus* and *V. vulnificus*.

### Characteristic features

Members of the genus *Vibrio* are characterized by the following:

1. Gram-negative curved bacilli. (Fig.114)

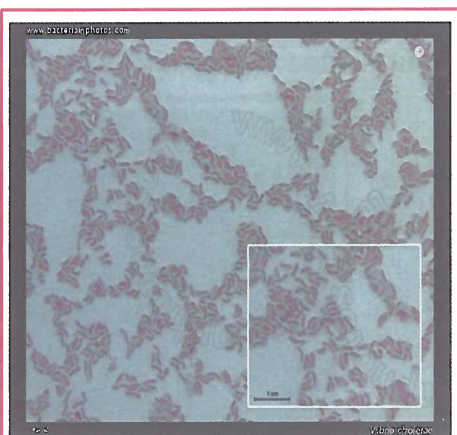


Fig.114: *Vibrio* (Gram stain)

2. Highly motile with a single polar flagellum (**darting motility**). (Fig.115)

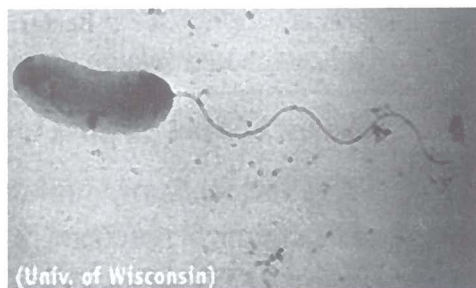


Fig.115: *Vibrio* with a single polar flagellum

3. Facultative anaerobes (preferring oxygenated environment).  
4. Tolerant to alkaline conditions and destroyed by low pH.  
5. Ferment sugars without gas production and reduce nitrates.  
6. **Oxidase-positive** (therefore, not classified as *Enterobacteriaceae*). (Fig.116)  
7. **String-positive**.

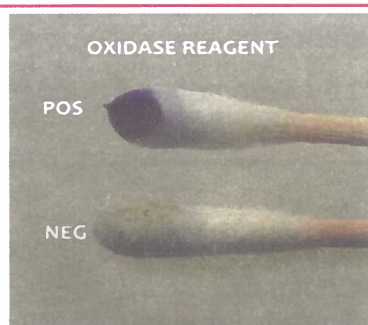


Fig.116: *Vibrio cholera*: oxidase positive

### String test (Fig.117)

- The string test differentiates vibrios (string-positive) from *Aeromonas* (string-negative).
- The test is done by emulsifying a large colony in a small drop of 0.5% sodium desoxycholate. Within 60 seconds, the cells lyse and DNA strings can be observed when a loopful is lifted from the slide up to 3 cm.

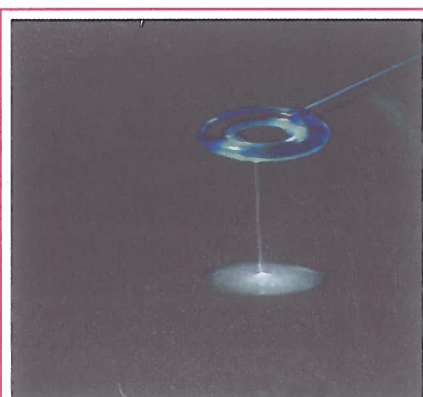


Fig.117: String test (positive)

## *Vibrio cholerae*

### Morphology (mentioned above)

### Cultural characters

- *V. cholerae* is a facultative anaerobe and grows best under alkaline conditions.
- It can be grown on:
  - Alkaline peptone water (pH 8.5): a surface pellicle, containing the organism, is formed after an incubation period of 6 to 8 h at 37°C. (Fig.118)
  - Nutrient agar at slightly alkaline pH.
  - Thiosulphate citrate bile sucrose (TCBS) agar (a selective indicator medium). *V. cholerae* produces yellow colonies on TCBS owing to sucrose fermentation. (Fig.119)

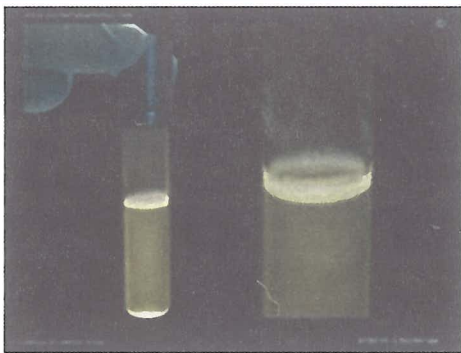


Fig.118: *V. cholerae* in alkaline peptone (surface pellicle)



Fig.119: *Vibrio cholerae* on T.C.B.S.

### Biochemical reactions (Fig.120)

- *Vibrio cholerae* ferments glucose, maltose, mannite and sucrose without gas production.
- On TSI, it gives acidic (yellow) butt and acidic (yellow) slant with no cracking of the agar.
- *V. cholerae* is oxidase-positive and indole-positive.



Glucose & lactose fermentation



Indole positive

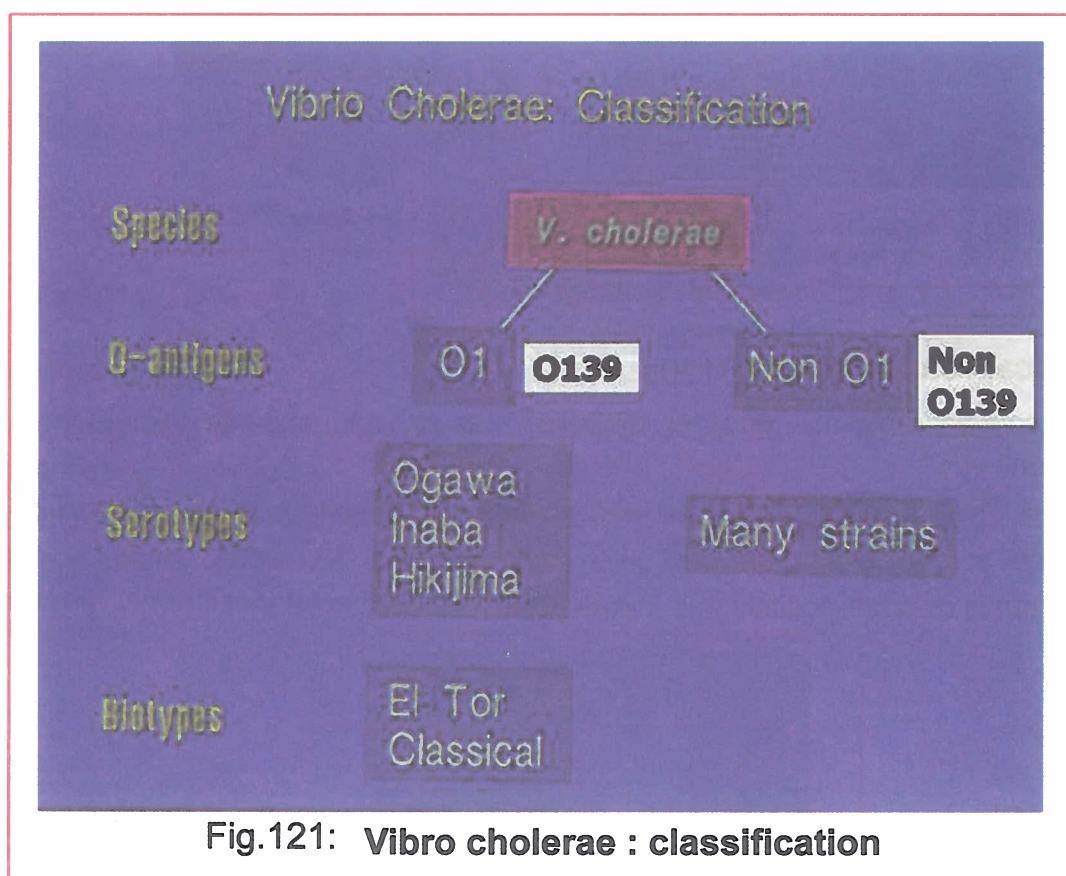
Fig.120: BR of *V. cholerae*



### Serological characters and biotypes of *V. cholerae*

- O antigens are specific and distinguish strains of *V. cholerae* into **serogroups** designated in numerals (O1, O2, O3, ..... etc).
- The serogroup O1 has three distinct **serotypes** (Ogawa, Inaba and Hikojima) based on antigenic differences.
- Each serotype may display the **classical** or **El Tor** biotype based on differences in biochemical reactions.
- *V. cholerae* serogroups **O1** and **O139** are the causative agents of **epidemic cholera**.
- Other serogroups (**non O1/O139**) are involved in sporadic forms of cholera-like diarrhoeal disease, but not in epidemics.

N.B.: The **flagellar (H) antigens** are shared with all vibrios and are, therefore, of no use in distinguishing strains. (Fig.121)



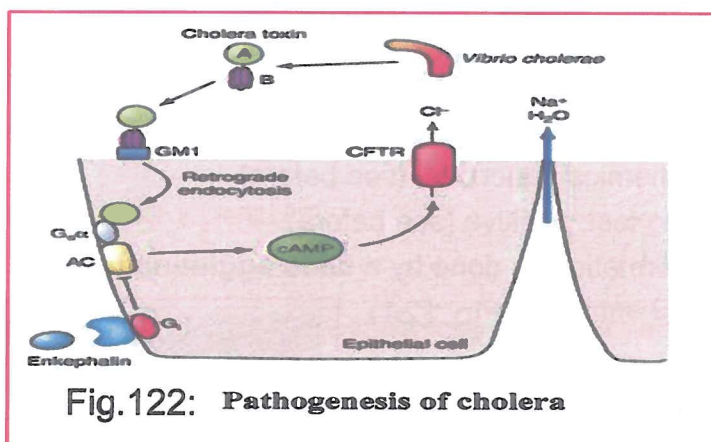
Cholera is a severe diarrhoeal disease caused by *V. cholerae* serogroups O1 or O139.

### Pathogenesis

- The natural reservoir of the organism is humans and may also be the aquatic environment.
- Infection is transmitted primarily by the faeco-oral route, through contaminated water or food.
- Vibrios are sensitive to acid and most die in the stomach. A high infectivity dose ( $10^7$ - $10^{12}$ ) is, therefore, required and this is why direct person-to-person spread is not common. Achlorhydria or antacids reduce the infective dose.
- Bacteria that passed the stomach penetrate the mucous layer of the small intestine and adhere to its mucosa by fimbriae and other colonization factors.
- *V. cholerae* will then multiply and secrete the potent cholera enterotoxin (choleragen).

### Cholera toxin (Fig.122)

- It is composed of 5 binding (B) subunits and an active (A) subunit.
- The toxin binds through its B subunits to specific receptors on the intestinal epithelial cells.
- The enzymatically active (A) subunit enters the cells and activates the adenylate cyclase enzyme causing a rise in cAMP production. This causes massive secretion of electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$ ) and water into the lumen of the small intestine.



### Clinical Manifestations

- Incubation period ranges from 1-4 days.
- The disease begins with an abrupt onset of vomiting and massive watery diarrhoea. The watery diarrhoea is speckled with flakes of mucus and epithelial cells (**rice-water stools**) and contains enormous numbers of vibrios. Several litres of fluid may be secreted within hours.(Fig.123)

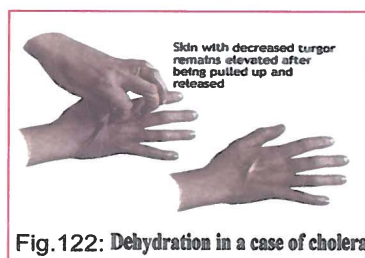


Fig.122: Dehydration in a case of cholera



- The disease runs its course in 2 to 7 days; the outcome depends upon the extent of water and electrolyte loss and the efficiency of treatment.
- Death may occur from hypovolaemic shock.
- Chronic carriers are rare although convalescent carriers may occur.

## Diagnosis

The diagnosis is suggested by the characteristic clinical picture. Patients with severe secretory diarrhoea must receive fluid and electrolyte replacement immediately regardless of aetiology. Full bacteriologic diagnosis is essential to apply the required infection control measures and to notify the authorities.

### A. Specimens

Stools (or rectal swabs for carriers).

### B. Cultivation

Faecal specimens should be inoculated directly on both:

- Differential selective solid media such as TCBS and MacConkey's agar media.
- Enrichment media such as alkaline peptone water. A surface pellicle is formed after an incubation of 6-8 h at 37°C. Subcultures, from the surface pellicle, are done on TCBS and incubated overnight at 37°C.

### C. Identification

- *V. cholerae* yields:
  - Yellow (sucrose-fermenting) colonies on TCBS medium.
  - Pale (lactose-nonfermenting) colonies on MacConkey's agar.
- Microscopic examination of:
  - A wet mount: shows rods with darting motility.
  - A Gram-stained smear: shows small, Gram-negative curved rods.
- Biochemical reactions: (see before).
- String test: positive (see before).
- Confirmation: is done by a **slide agglutination test** with specific anti-O1 and anti-O139 antisera. (Fig.124)

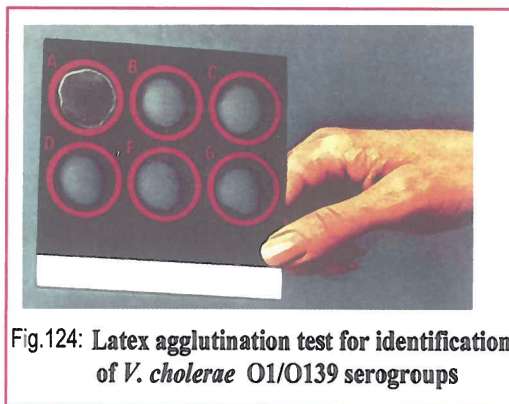


Fig.124: Latex agglutination test for identification of *V. cholerae* O1/O139 serogroups

N.B.: During an epidemic, diagnosis depends mainly on clinical judgment, and there is little need for the laboratory. Laboratory confirmation may be done by microscopical examination of a wet mount of liquid stools to detect the characteristic darting motility of vibrios which is stopped by specific antibody.



## Treatment

- Treatment of cholera consists **essentially** of replacing fluid and electrolytes either intravenously or orally.
- Antibiotics such as tetracycline or ciprofloxacin are useful in treatment. They shorten duration of diarrhoea and reduce the time of excretion of organisms.

## Control

1. **Sanitation:** Application of sanitary principles that protect drinking water and food from contamination with human faeces.
2. **Vaccination:** Three vaccines are available (Table 6).

**Table (6):** Cholera vaccines

Nature of vaccine	Route	Protection duration
Killed cholera vaccine	Intramuscular	3-6 months
Live-attenuated vaccine	Oral	At least 6 months
Whole cell/B subunit vaccine	Oral	2 years

### N.B.:

- Oral vaccines provide somewhat better protection.
- An inexpensive, effective cholera vaccine that provides long-term protection is not yet available.

3. **Chemoprophylaxis:** Tetracycline may be given to close contacts but it cannot prevent the spread of an epidemic.

## Other *Vibrio* species

**Table (7):** Other *vibrio* species

Species	Reservoir	Disease	Transmission	Manifestations	Treatment
<i>Vibrio para-haemolyticus</i>	Sea water*	Gastro-enteritis	Consumption of undercooked or raw seafood	Watery diarrhoea with abdominal cramps	Self- limiting
<i>V. vulnificus</i>	As above	Gastro-enteritis	As above	As above	As above
		Cellulitis	Swimming in sea water	Rapidly spreading lesions	Tetracycline; 3 <sup>rd</sup> generation cephalosporins

\* Halophilic (salt-loving) organisms

## Aeromonas

- *Aeromonas* species are morphologically similar to vibrios.
- They are distinguished from vibrios by being **string-negative**.
- They are widespread inhabitant in water and soil.
- *Aeromonas hydrophila* is the most important species. It causes:
  - Wound infections.
  - Diarrhoea resembling cholera.
  - Bacteraemia and septicaemia in immunocompromised patients.

### MCQs:

- 1- The yellow colonies of *V. cholerae* on TCBS agar medium is due to:
  - a- Endopigment production
  - b- Exopigment production
  - c- Sucrose fermentation
  - d- Lactose fermentation
  - e- Nitrate reduction
- 2- Cholera toxin:
  - a- Acts by inhibition of protein synthesis
  - b- Acts by inhibition of acetyl choline release
  - c- Is secreted by lysogenic strains only
  - d- Is classified as an enterotoxin
  - e- Has 5 A and 1 B subunits
- 3- An individual experiences diarrhoea after eating raw shell fish. What is the most probable cause?
  - a- *Campylobacter jejuni*
  - b- *Salmonella Choleraesuis*
  - c- *Shigella dysenteriae*
  - d- *Vibrio parahaemolyticus*
  - e- *Yersinia enterocolitica*
- 4- *V. cholerae* can be distinguished from *Aeromonas hydrophila* by:
  - a- Gram stain
  - b- Motility
  - c- Natural habitat
  - d- Oxidase test
  - e- String test

## CAMPYLOBACTER

### ILOs:

By the end of this chapter the student should be able to:

- Identify the characteristic features of *Campylobacter*
- Describe the culture characteristics & growth requirements of *Campylobacter jejuni*
- Describe the pathogenesis and clinical features of campylobacter enteritis
- Describe laboratory diagnostic methods for campylobacter enteritis
- List treatment and means of prevention of campylobacter enteritis

Campylobacters are carried in the intestinal tract of a wide variety of birds (especially poultry) and domestic animals (e.g. cattles, dogs and cats).

### Characteristic features

1- They are curved or S-shaped, slender, Gram-negative rods. (Fig.125)

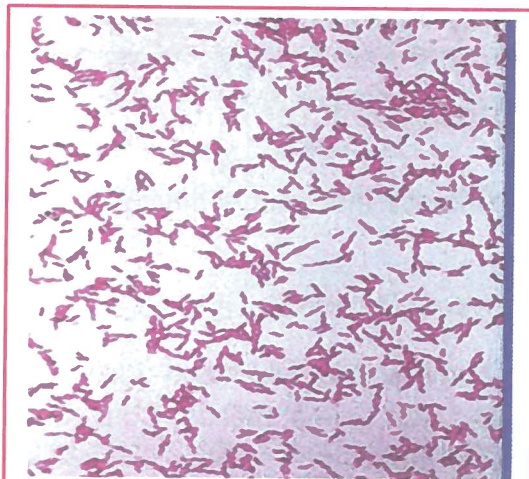


Fig.125: *Campylobacter* (Gram stain)

- 2- They have single polar flagella contributing to the characteristic darting motility.
- 3- Most campylobacters are microaerophilic (requiring 5% O<sub>2</sub> and 10% CO<sub>2</sub>).
- 4- They are oxidase positive.

The genus comprises many species, the most important of which is *C. jejuni* which accounts for about 90% of the *Campylobacter* diseases, especially in children.



## *Campylobacter jejuni*

### Morphology (mentioned above)

**Cultural characters:** *C. jejuni* can be grown on:

- Enriched media e.g. blood or chocolate agar.
- Selective media e.g. Skirrow's medium which is blood agar that is rendered selective by addition of antibiotics.

Cultures are incubated in microaerophilic atmosphere at 42°C (thermophilic) for 2 days. (Fig.126)



Fig.126: *Campylobacter* on Skirrow's medium

## *Campylobacter enteritis*

*Campylobacter jejuni* causes enteritis that affects mainly children under 5 years and young adults.

### Transmission

- *C. jejuni* is transmitted to humans primarily faeco-orally, through consumption of food and water contaminated with animal faeces. Common foods include poultry, raw milk, meat, fruits and vegetables.
- Exposure to sick kittens and puppies has also been associated with outbreaks especially in children.
- Person-to-person transmission may rarely occur.

### Pathogenesis

- *C. jejuni* adheres to intestinal epithelial cells or overlying mucus.
- It invades and destroys epithelial cells in the jejunum, ileum and colon.
- Some strains of *C. jejuni* produce an enterotoxin (similar to cholera toxin) that causes watery diarrhoea, and a cytotoxin that causes bloody diarrhoea.

## Clinical manifestation

- The incubation period is 2-5 days.
- The disease begins as watery diarrhoea followed by bloody stools accompanied by fever and abdominal cramps.
- Most infections are self-limited and illness generally lasts 7-10 days.
- *Campylobacter* enteritis may be complicated by any of the following autoimmune diseases:
  - **Guillain-Barré syndrome:** It is an acute paralytic disease of the peripheral nervous system. It is attributed to antibodies against *C. jejuni*, that cross react with antigens on neurons.
  - Reactive arthritis.
  - Reiter's disease: It is characterized by the triad of arthritis, conjunctivitis and urethritis. Most patients are men who are HLA-B27-positive.

## Laboratory diagnosis

**A. Specimens:** Stools.

**B. Direct detection:** Presumptive diagnosis can be made by finding curved organisms with rapid darting motility in a wet mount of faeces.

**C. Cultivation:** *C. jejuni* can be isolated from faecal specimens by using:

- A filtration method and a non-selective culture medium. A bacterial filter with a certain pore size is used. It permits passage of the small slender campylobacters and excludes larger faecal organisms. The filtrate is then inoculated on a non-selective medium e.g. chocolate agar.
- A selective medium (e.g. Skirrow's medium).

In either method, cultures are incubated in a microaerophilic atmosphere at 42°C for 2 days.

**D. Identification:** Growth of *C. jejuni* is identified biochemically.

## Treatment

- Replacement of fluids and electrolytes is the main line of therapy.
- Erythromycin is the antibiotic of choice.

## Prevention

- Proper cooking of chicken, pasteurization of milk and chlorination of drinking water.
- Proper sewage disposal.
- Personal hygiene (especially hand washing).

**MCQs:**

The following 2 questions are related:

A 17-year-old man had a grilled chicken dinner with his friends. Three days later he went to the hospital with severe abdominal cramps; stools were bloody and pus cells were present. Culture revealed Gram-negative, oxidase positive, S-shaped rods.

- 1- The most probable causative agent is:
  - a- *Vibrio cholerae*
  - b- *Salmonella Typhimurium*
  - c- *Campylobacter jejuni*
  - d- *Shigella flexneri*
  - e- *Helicobacter pylori*
  
- 2- The special laboratory conditions required to isolate this organism are:
  - a- 37°C, aerobic on TCBS agar plates
  - b- 42°C, microaerophilic on Skirrow's medium
  - c- 37°C, microaerophilic on MacConkey's medium
  - d- 37°C, anaerobic on blood agar plates
  - e- 42°C, aerobic on Skirrow's medium
  
- 3- True or false:
  - a- Campylobacters have multiple polar flagella.
  - b- Campylobacters have a characteristic darting motility.
  - c- Like *Enterobacteriaceae*, *Campylobacter jejuni* is oxidase negative.
  - d- Campylobacter enteritis may be complicated by reactive arthritis.
  - e- Skirrow's medium is rendered selective by addition of tellurite salts.
  - f- Sick kittens and puppies are responsible for outbreaks of campylobacter infections in children.
  - g- *Campylobacter jejuni* can be isolated from faecal specimens by filtration.
  - h- Campylobacter enteritis can be prevented by a live attenuated oral vaccine.



## HELICOBACTER

### ILOs:

**By the end of this chapter the student should be able to:**

- Identify the characteristic features of *Helicobacter*
- Describe the culture characteristics & growth requirements of *H. pylori*
- Outline important virulence factors of *H. pylori*
- Describe the pathogenesis and list diseases caused by *H. pylori*
- Describe laboratory diagnostic methods for infections due to *H. pylori*
- Outline the treatment regimen of *H. pylori* infection

Helicobacters (particularly *Helicobacter pylori*) are the only organisms which are able to colonize the stomach. *H. pylori* infections are relatively common and worldwide in distribution.

### Characteristic features

- Helicobacters are similar to campylobacters in the following:
  1. Spiral or curved Gram-negative bacilli. (Fig.127)
  2. Able to grow on the same media (e.g. Skirrow's medium). (Fig.128)

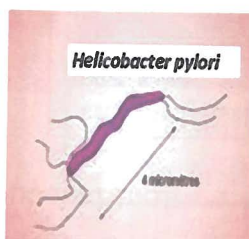
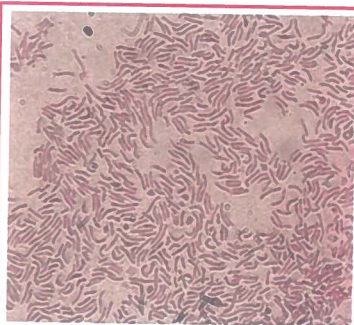


Fig.127: *Helicobacter pylori*



Fig.128: Skirrow's medium

3. Microaerophilic.
  4. Oxidase-positive.
- Helicobacters differ from campylobacters in the following:
    1. They have multiple polar flagella contributing to the characteristic corkscrew movement.
    2. They can grow at 37°C but not at 42°C.

*H. pylori* is the most important *Helicobacter* species since it is the causative agent of peptic ulcer. The latter is now approached as an infectious disease.

## *Helicobacter pylori*

### Morphology (mentioned above)

### Cultural characters

- *H. pylori* can be grown on:
  - Enriched media e.g. blood or serum agar.
  - Selective media e.g. Skirrow's medium.
- The organism requires **microaerophilic** condition, high humidity and incubation at 37°C for 3-6 days.

### Biochemical reactions

- All helicobacters are oxidase positive.
- *H. pylori* produces large quantities of **urease**.

### Epidemiology and transmission

- *H. pylori* has been found in the stomach of humans in all parts of the world. There appears to be no reservoir of *H. pylori* aside from the human stomach. (Fig.129)



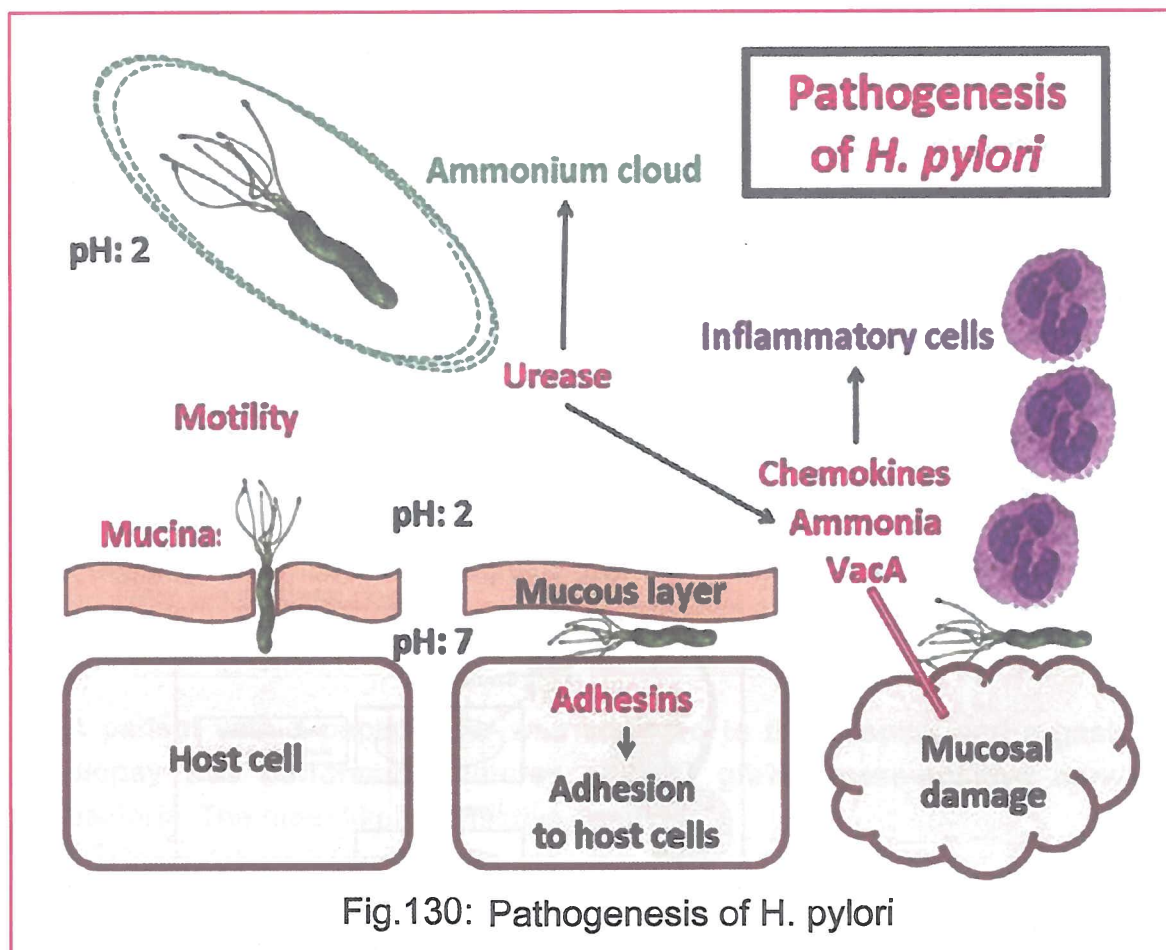
Fig.129: *H. pylori* in gastric mucosa

- Transmission of *H. pylori* is thought to be from person to person (faeco-oral or oral-oral) as evidenced by the following:
  - There is clustering of infection within families.
  - The organism has not been isolated from food or water.

### Pathogenesis

- **Urease** production, **motility** and **mucinase** are essential for colonization:
  - Urease cleaves urea producing "ammonium cloud", which permits *H. pylori* to survive in an acidic environment.
  - The corkscrew motility enables the organism to penetrate (pore) through the viscous gastric mucus.
  - Mucinase helps in penetration of mucous layer by the organism allowing it to reach the stomach lining, where the pH is neutral.

- The organism binds to the mucosal cells by **adhesins**.
- Mucosal damage occurs as a result of:
  - Release of **ammonia**.
  - Production of **vacuolating cytotoxin (VacA)**.
  - Recruitment and activation of **inflammatory cells**. This is mediated by chemokines (e.g. interleukin-8), the production of which is controlled by genes within a pathogenicity island. (Fig.130)

Fig.130: Pathogenesis of *H. pylori*

### Clinical outcomes

- **Chronic gastritis:** It develops in colonized people and may be associated with epigastric discomfort.
- **Duodenal or gastric ulcers:** *H. pylori* is associated with >90% of duodenal ulcers and 70-80% of gastric ulcers.
- **Stomach cancer:** Gastric carcinoma or gastric lymphoma may follow the chronic gastritis. *H. pylori* is now classed by the WHO as type I carcinogen.

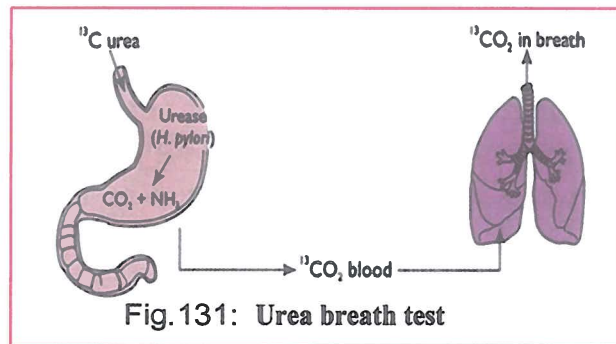
### Diagnosis

#### I. Non invasive methods:

1. **Antigen detection:** Detection of *H. pylori* antigens in stools is done by ELISA for diagnosis and follow up of treatment.



2. **Urea breath test:** Radiolabeled urea is ingested. If the organism is present, urease will cleave the ingested urea, releasing radiolabeled  $\text{CO}_2$  which can be detected in the breath. (Fig.131)



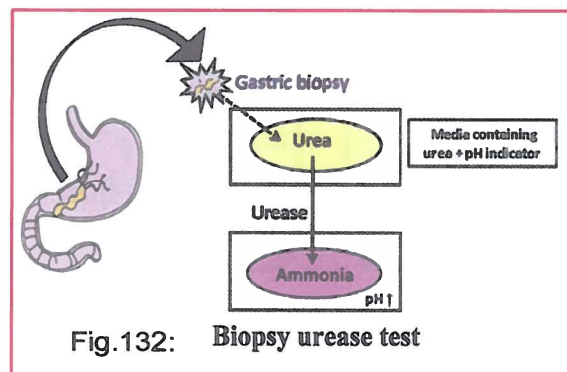
3. **Serology:** The presence of IgG antibodies in the patient's serum can be used as evidence of infection.

## II. Invasive methods:

**A. Specimens:** Gastric biopsies obtained by endoscopy.

### B. Direct detection

- 1- **Histologic examination:** *H. pylori* can be visualized in the biopsy sections using special stains.
- 2- **Biopsy urease tests:** The gastric biopsy is placed onto a urea-containing medium with a pH indicator which changes its colour upon release of ammonia. (Fig.132)



- 3- **Molecular methods:** DNA probes and PCR can also be used to detect *H. pylori* nucleic acid.

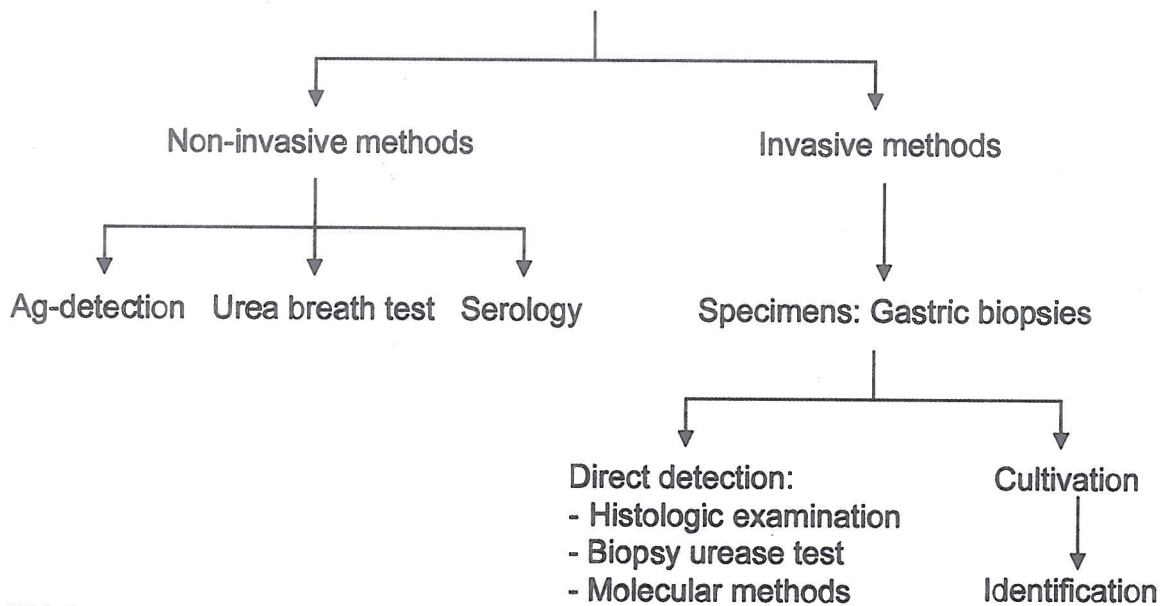
### C. Cultivation (mentioned above)

### D. Identification

- 1- *H. pylori* is oxidase positive and **strong urease** producer.
- 2- It can also be identified by DNA probes and PCR.

## Treatment

- Elimination of *H. pylori* requires combination therapy with 2 antibiotics and an antacid.
- A typical regimen includes amoxicillin plus clarithromycin plus a proton-pump-inhibitor.

**Diagnosis of *H. pylori* infections****MCQs:**

- 1- ***Helicobacters* are similar to campylobacters in the following EXCEPT:**
  - a- They are curved Gram-negative bacilli.
  - b- They are microaerophilic.
  - c- They can grow at 42°C.
  - d- They are oxidase positive
  - e- They are able to grow on Skirrow's medium.
- 2- **A patient with a peptic ulcer was admitted to the hospital and a gastric biopsy was performed. Cultures at 37°C grew urease-positive curved bacteria. The most likely causative agent is:**
  - a- *Campylobacter jejuni*
  - b- *Vibio parahaemolyticus*
  - c- *Haemophilus influenzae*
  - d- *Shigella dysenteriae*
  - e- *Helicobacter pylori*
- 3- **A patient has a gastric ulcer caused by *H. pylori*. Which characteristic appears to play the most central role in the ability of the organism to colonize the stomach?**
  - a- Oxidase production
  - b- Urease production
  - c- Microaerophilic lifestyle
  - d- O-antigens
  - e- Motility

## NON-FERMENTATIVE GRAM-NEGATIVE BACILLI

### ILOs:

**By the end of this chapter the student should be able to:**

- List characteristic features of *Pseudomonas* species and *Acinetobacter* species and describe how they are distinguished from each other
- Recognize the various habitats of *Pseudomonas aeruginosa*
- Describe the culture characteristics of *P. aeruginosa*
- Outline important virulence factors of *P. aeruginosa*
- Describe the pathogenesis and list diseases caused by *P. aeruginosa*
- Recognize the importance of *Acinetobacter* species in hospital-acquired infections and list diseases caused by them

### *Pseudomonas*

#### Characteristic features

1. Non-fermentative, motile, Gram-negative bacilli. (Fig.133)
2. Aerobic.
3. Most species are oxidase positive. (Fig.134)

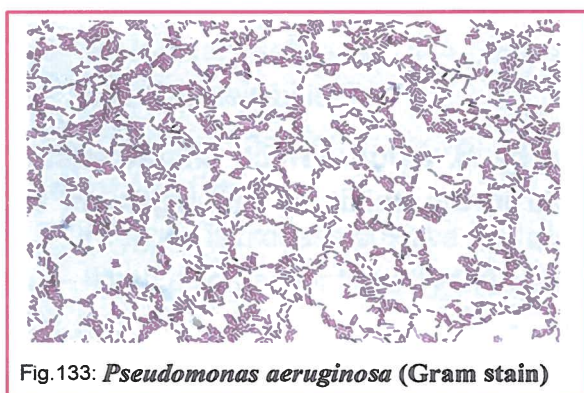


Fig.133: *Pseudomonas aeruginosa* (Gram stain)

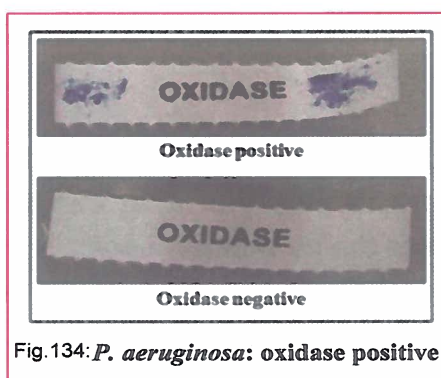


Fig.134: *P. aeruginosa*: oxidase positive

*Pseudomonas aeruginosa* is the most important species.



## *Pseudomonas aeruginosa*

*P. aeruginosa* is worldwide in distribution preferring moist environments:

- It is found in water, soil and on plants.
- It may be part of the normal microbial flora, particularly in the gastrointestinal tract and moist body sites.
- It is able to grow in water containing only traces of nutrients, e.g. tap water. It also has a remarkable ability to resist disinfectants. These factors favour its persistence in the hospital environment.

### Morphology (mentioned above)

### Cultural characters

- *P. aeruginosa* grows on:
  - Nutrient agar: It usually produces diffusible exopigments. When the yellow pyoverdine pigment combines with the blue pyocyanin pigment, the bright green colour characteristic of *P. aeruginosa* colonies is created. (Fig.135)
  - Blood agar: It causes complete haemolysis.
  - MacConkey agar: It produces pale lactose non-fermenting colonies. (Fig.136)
  - TSI: It gives red (alkaline) butt and red (alkaline) slant. (Fig.137)
- It has a grape-like (fruity) odour.



Fig.135: *Pseudomonas aeruginosa* on nutrient agar (showing greenish exopigment)

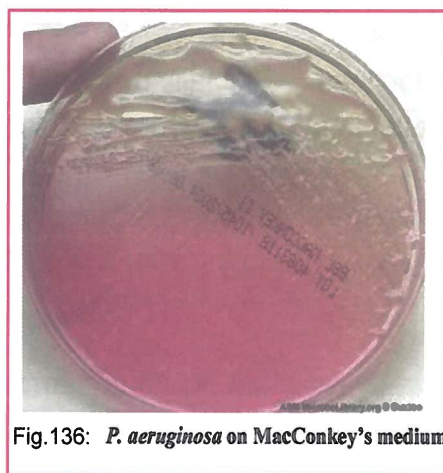


Fig.136: *P. aeruginosa* on MacConkey's medium

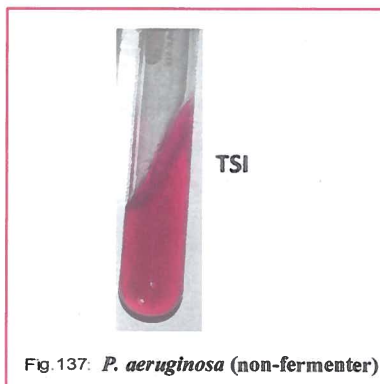


Fig.137: *P. aeruginosa* (non-fermenter)

### Biochemical activities

- *P. aeruginosa* is oxidase-positive.
- It is carbohydrate non-fermenter; acid is produced from glucose only oxidatively.

### Pathogenesis

*P. aeruginosa* is a significant human pathogen particularly in immunocompromised patients. It is a major cause of hospital-acquired (nosocomial) infections. The organism is considered invasive and toxigenic and may cause pyogenic infections.

**Virulence factors** include:

- Pili.
- Exotoxin A: It causes tissue necrosis by the same mechanism as diphtheria exotoxin.
- Enzymes: Proteases and elastases.
- Endotoxin.
- Capsule: The polysaccharide material allows the organism to live in a biofilm away from antibodies and phagocytes. Capsulated strains predominate in patients with cystic fibrosis.

### Diseases

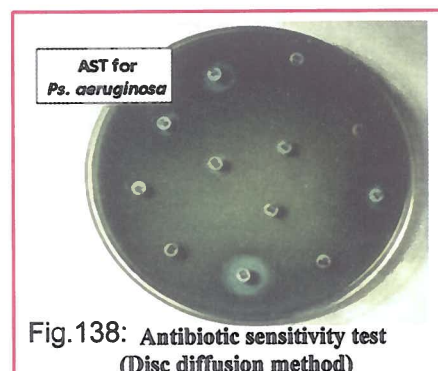
*P. aeruginosa* can cause infections virtually anywhere in the body:

- 1- Urinary tract infections (UTI).
- 2- Respiratory tract infections (RTI): especially in intubated patients or in those with cystic fibrosis.
- 3- Wound infections (especially burns).
- 4- External ear infections: in swimmers (swimmer's ear), diabetics and the elderly.
- 5- Folliculitis.
- 6- Eye infections: frequently associated with the use of contact lens.

N.B.: UTI, RTI and wound infections predominate in hospitals.

### Treatment

*P. aeruginosa* is resistant to many antibiotics. Combined antibiotic treatment is usually indicated in serious infections (anti-pseudomonal  $\beta$ -lactam e.g. piperacillin plus an aminoglycoside e.g. gentamycin). (Fig.138)



## Acinetobacter

- *Acinetobacter* species are similar to *P. aeruginosa* in being non-fermentative, aerobic, Gram-negative bacilli. (Fig.139)
- They differ from *P. aeruginosa* in being non-motile, and **oxidase-negative**.
- They are free-living saprophytes found in soil, water and foods. They can be found as commensals on the skin.
- They are opportunistic pathogens causing **hospital-acquired infections**, particularly in ICUs. Antibiotic resistant strains may be responsible for outbreaks of pneumonia, UTI and wound infections that may progress to septicaemia.

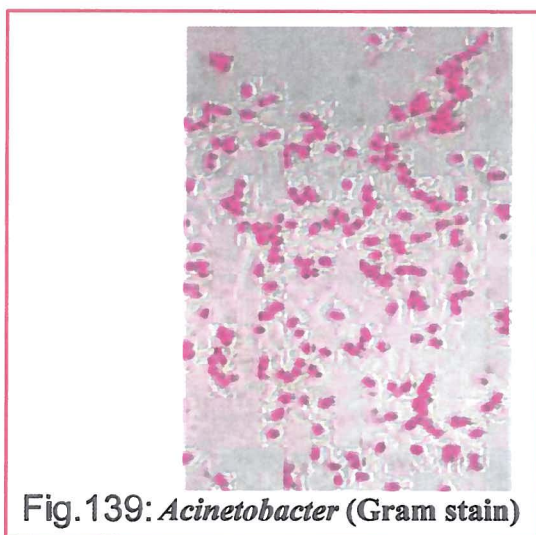


Fig.139: *Acinetobacter* (Gram stain)

### MCQs:

- 1- *P. aeruginosa* is characterized by one of the following features:
  - a- It gives rose pink colonies on MacConkey's medium.
  - b- It ferments many sugars with production of acid only.
  - c- It is a facultative anaerobe.
  - d- It produces diffusible exopigments.
  - e- It is a spore-forming bacillus.
- 2- A 65-year-old man develops dysuria and haematuria. A gram stain of a urine sample shows gram-negative rods. Culture on MacConkey agar reveals lactose non-fermenting colonies without swarming. Which one of the following organisms may be the cause of this condition?
  - a- *Enterococcus faecalis*
  - b- *Pseudomonas aeruginosa*
  - c- *Proteus vulgaris*
  - d- *Escherichia coli*
  - e- *Klebsiella pneumoniae*



## HAEMOPHILUS

### ILOs:

#### By the end of this chapter the student should be able to:

- Identify the characteristic features of *Haemophilus* species and list medically important species
- Describe the culture characteristics & growth requirements of *H. influenzae*
- Outline important virulence factors of *H. influenzae*
- Demonstrate the pathogenesis and list diseases caused by *H. influenzae*, *H. ducreyi* and *H. aegyptius*
- Describe laboratory diagnostic methods of infections due to *H. influenzae*
- Recognize the measures for prevention of diseases caused by *H. influenzae*

The genus name is derived from the Greek words meaning "blood-loving".

### Characteristic features

#### 1. Gram-negative bacilli (Fig.140)

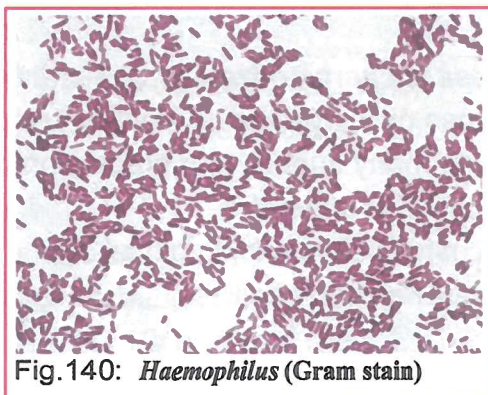


Fig.140: *Haemophilus* (Gram stain)

2. Pleomorphic, ranging from coccobacilli to long slender filaments
3. Facultative anaerobes
4. Require one or both of the following growth factors which are normally found in blood:
  - **X factor** (haemin): is available from non-haemolysed as well as haemolysed RBCs.
  - **V factor** (NAD): is liberated from lysed RBCs.

### Medically important *Haemophilus* species

1. *H. influenzae*
2. *H. ducreyi*
3. *H. aegyptius* (*H. influenzae* biotype *aegyptius*)

### *Haemophilus influenzae*

*H. influenzae* occurs as part of the normal flora of the upper respiratory tract. It is so-called because it was thought to be the causative agent of influenza.

### Morphology

- Pleomorphic Gram-negative bacilli.
- Some strains have a polysaccharide capsule.

### Cultural characters (Fig.141,142,143)

- *H. influenzae* requires both X and V factors for growth. Therefore, it can be cultured on:

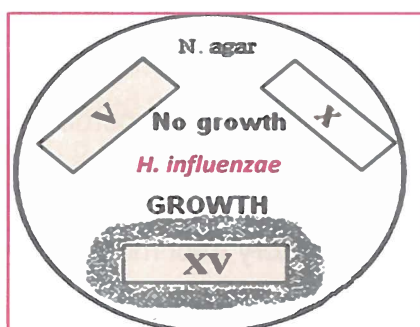


Fig.141: *Haemophilus influenzae*  
(growth only around both factors X & V)

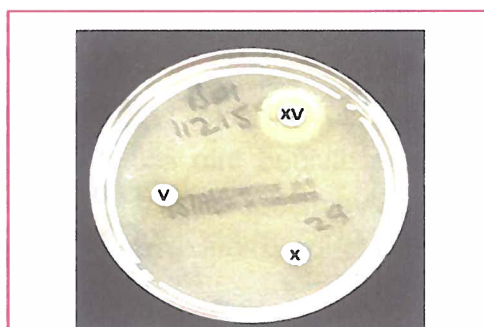


Fig.142: *Haemophilus influenzae*  
(growth only around both factors X & V)

- Chocolate agar (heat-lysed blood). The heat used in preparing chocolate agar:
  1. provides V factor and extra X factor from RBCs into the medium.
  2. inactivates non-specific inhibitors (e.g. NADase).
- Blood agar provided with free V factor.

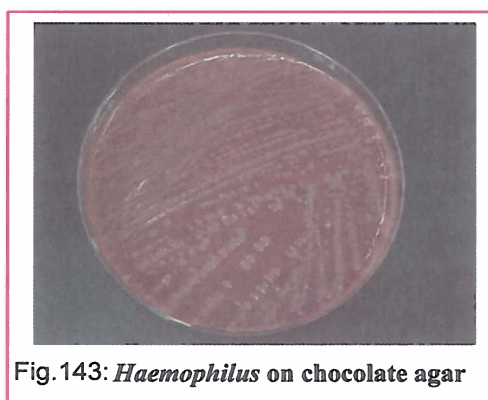


Fig.143: *Haemophilus* on chocolate agar

- **Satellatism:** Growth of *H. influenzae* on blood agar around colonies of a haemolytic organism (e.g., *S. aureus*) that liberates V factor from lysed RBCs. (Fig.144)
- Growth is enhanced by 5% CO<sub>2</sub>. (Fig.145)

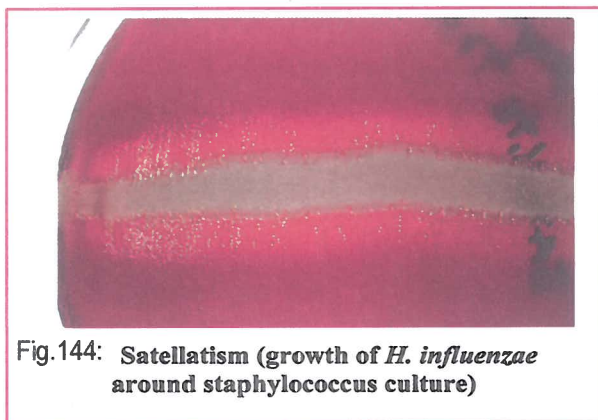


Fig.144: Satellatism (growth of *H. influenzae* around staphylococcus culture)



Fig.145: Candle Jar (5 – 10% CO<sub>2</sub>)

### Virulence factors and clinical significance

- 1- The major virulence factor is the **polysaccharide capsule**, according to which *H. influenzae* is divided into six serotypes a-f. Type b (Hib) causes most of the severe invasive diseases.
- 2- IgA protease helps colonization of the upper respiratory tract mucosa.

### Pathogenesis

- *H. influenzae* is transmitted by respiratory droplets, resulting in either:
  1. Asymptomatic colonization of the upper respiratory tract. Colonization is facilitated by IgA protease production.
  2. Infections (mainly by **non-capsulated strains**) such as otitis media, sinusitis, epiglottitis or pneumonia. Pneumonia occurs mainly following viral infection or in patients with chronic lung disease.
- Blood stream invasion (mainly by **capsulated strains**): After being established in the upper respiratory tract, the organism can enter the blood stream (bacteraemia) and spread to the meninges (meningitis).
- Most infections occur in children between the ages of 6 months and 6 years. This is attributed to the decline of maternal IgG together with the inability of the child to generate antibodies against the polysaccharide capsular (TI) antigen.



## Laboratory diagnosis

**A. Specimens** include CSF, blood, pus and respiratory secretions

### B. Direct detection

1. Gram-stained smear: Gram-negative coccobacilli associated with PMNLs.
2. Quellung reaction.
3. Detection of type b capsular antigen in CSF by latex agglutination or fluorescent antibody test.
4. PCR.

### C. Cultivation

1. Specimens other than the blood should be plated directly onto chocolate agar and incubated at 37°C with 5% CO<sub>2</sub>.
2. Blood samples should be cultivated by the blood culture technique. Subcultures are plated on chocolate agar and incubated as mentioned above.

### D. Identification

1. Colony morphology:
  - a- Noncapsulated strains produce small flat colonies.
  - b- Capsulated strains produce larger mucoid colonies.
2. Gram-stained film: Gram-negative pleomorphic coccobacilli.
3. Serological identification by latex agglutination or Quellung reaction.
4. DNA probes.

## Treatment

Third generation cephalosporins (e.g., cefotaxime or ceftriaxone) are the antibiotics of choice.

## Prophylaxis

### 1. *H. influenzae* type b (Hib) vaccine:

- It is a capsular polysaccharide conjugated to a carrier protein (such as diphtheria toxoid) to elicit a T-dependent immune response. It provides protective immunity even in children younger than 24 months.
- Hib vaccine is given to children at the age of 2, 4, 6, and 15 months. It has reduced the incidence of serious infections caused by this organism.

### 2. Rifampicin is used for chemoprophylaxis of unvaccinated close contacts of cases of Hib meningitis.

### *Haemophilus ducreyi*

- *H. ducreyi* causes **chancroid** (soft sore) which is a sexually transmitted disease.
- Chancroid manifests as painful, soft genital ulcer accompanied by inguinal lymphadenopathy (syphilitic chancre is painless and hard). (Fig.146)



Fig.146: *H. ducreyi* (Chancroid)

- **Diagnosis** is usually clinical because this highly fastidious organism is difficult to be grown in the laboratory.
- **Treatment:** Ceftriaxone and erythromycin are effective.

### *Haemophilus aegyptius*

*H. aegyptius* (*H. influenzae* biogroup *aegyptius*) causes the following infections:

1. **Acute mucopurulent conjunctivitis:** *H. aegyptius* is the commonest bacterial cause of this condition, which is known as pink eye. (Fig.147)



Fig.147: *H. aegyptius* (Conjunctivitis)

2. **Brazilian purpuric fever:** It is life-threatening childhood illness caused by certain strains of *H. aegyptius*. It is characterized by purpura and shock.

#### **Treatment**

Topical tetracycline and chloramphenicol are effective for treatment.

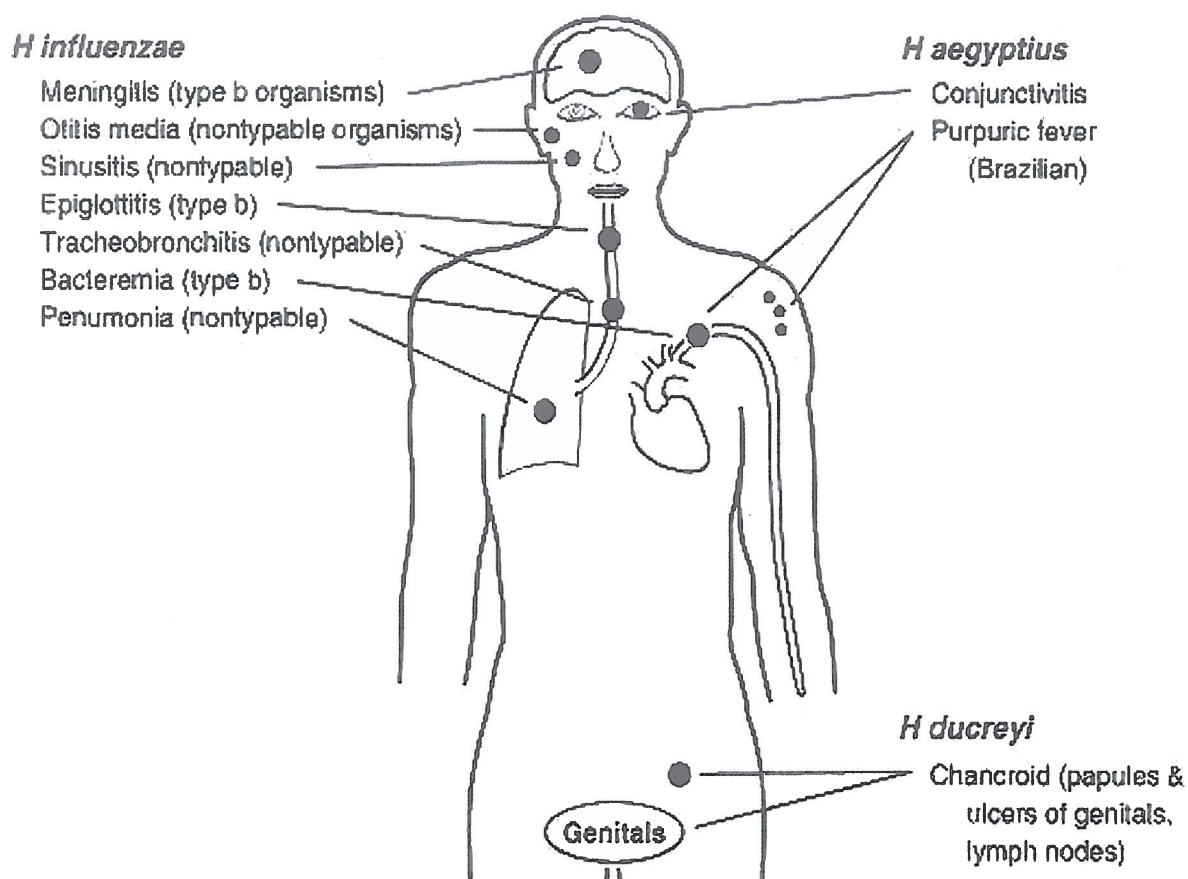


Fig.148: Diseases caused by Haemophilus

## MCQs:

- 1- The following statements refer to *H. influenzae* **EXCEPT**:
  - a- It is pleomorphic.
  - b- Its major virulence factor is IgA protease.
  - c- It requires both X-factor and V-factor for growth.
  - d- It is one of the major causes of meningitis.
  - e- It causes infection mainly in children between 6 months and 6 years of age.
  
- 2- *Haemophilus influenzae* type b:
  - a- Is a Gram-positive rod
  - b- Is a common cause of influenza
  - c- Can grow alone on blood agar
  - d- Has a polysaccharide capsule
  - e- Is the most common cause of urinary tract infection



- 3- The vaccine against *H. influenzae* is:
- a- Live attenuated *H. influenzae*
  - b- Killed *H. influenzae*
  - c- Toxoid derived from *H. influenzae*
  - d- Polysaccharide derived from *H. influenzae*
  - e- Polysaccharide derived from *H. influenzae* conjugated to a protein
- 4- A 2-year-old child came to the emergency room with high fever and breathing difficulty. Clinical investigation revealed inflammatory swelling of the epiglottis. Which of the following organisms is the most likely causative organism?
- a- *Haemophilus influenzae*
  - b- *Haemophilus ducreyi*
  - c- *Haemophilus aegyptius*
  - d- *Bordetella pertussis*
  - e- *Neisseria meningitidis*
- 5- Chancroid is characterized by the following **EXCEPT**:
- a- It is caused by *Haemophilus ducreyi*.
  - b- It is a sexually-transmitted disease.
  - c- It manifests as a genital ulcer.
  - d- It is hard and painless.
  - e- It is accompanied by enlarged inguinal lymph nodes.

## BORDETELLA

### ILOs:

By the end of this chapter the student should be able to:

- Describe the morphology and culture characters of *Bordetella pertussis*
- Outline the various factors that determine the virulence of *B. pertussis*
- Discuss the pathogenesis of pertussis
- Outline the laboratory diagnosis of pertussis
- Discuss measures of prevention of pertussis

### Characteristic features

1. Small, encapsulated Gram-negative coccobacilli. (Fig.149)
2. Strict aerobes.

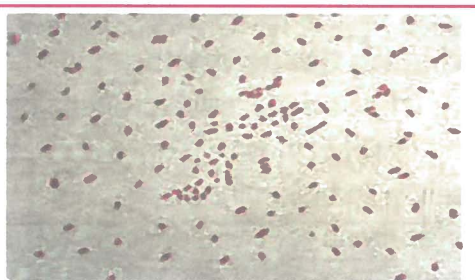


Fig.149: *Bordetella* (Gram stain)

*B. pertussis* is the most important species of the genus and causes whooping cough (pertussis).

### *Bordetella pertussis*

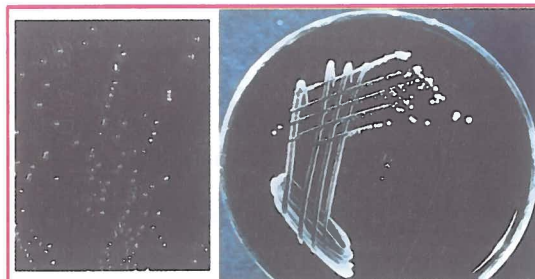
#### Morphology (mentioned above)

#### Cultural characters

- *Bordetella pertussis* can be grown on:
  - Bordet-Gengou medium (blood agar supplemented with special growth factors).
  - Charcoal blood agar.
- Growth is slow and requires incubation for 5-7 days at 37°C. (Fig.150)
- Colonies have characteristic mercury-droplet appearance. (Fig.151)

Fig.150: **BORDETELLA PERTUSSIS**

Named Bordet &amp; Gengou

Fig.151: *Bordetella* on charcoal blood agar medium (mercury droplet- appearance)

## Virulence Factors

### I- Factors mediating attachment (colonization):

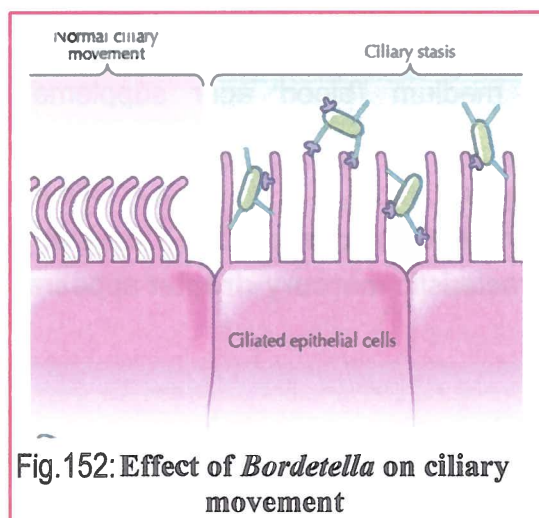
1. Filamentous haemagglutinin (FHA): It is a filamentous protein on the cell surface.
2. Pertussis toxin (PTx): It occurs in 2 forms:
  - cell-bound form acting as an adhesin.
  - secreted form acting as a toxin (see below).

### II- Factors (toxins) mediating tissue damage:

1. Pertussis toxin (PTx): It has an A-B structure/function model.
  - It causes a striking lymphocytosis.
  - It increases sensitivity to histamine.

Thus, pertussis toxin is an important virulence factor mediating both colonization and tissue damage.

2. Adenyl cyclase toxin:
  - It impairs leucocyte chemotaxis, thus, inhibiting phagocytosis.
  - It causes local oedema.
3. Tracheal cytotoxin: (Fig.152)
  - It interferes with ciliary movement.
  - It kills ciliated respiratory cells.
4. Endotoxin.

Fig.152: Effect of *Bordetella* on ciliary movement



## Whooping Cough (Pertussis)

It is an acute respiratory disease of childhood transmitted by droplet. (Fig.153)

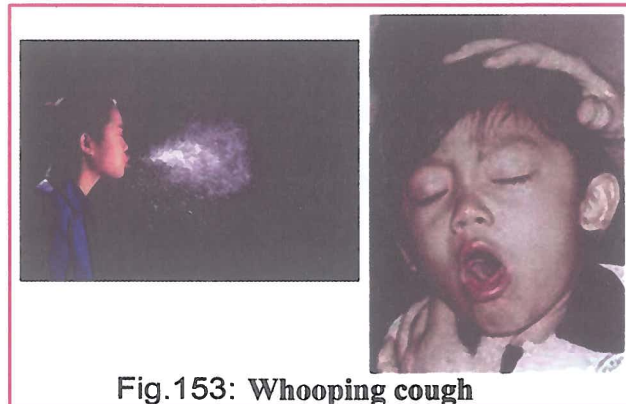


Fig.153: Whooping cough

Classical pertussis has 3 stages: the catarrhal, paroxysmal and convalescent stages.

**Table (8):** Stages of pertussis

	Incubation period	Catarrhal stage	Paroxysmal stage	Convalescent stage
Duration (weeks)	1-2	1-2	~3	~3
Virulence factors involved	FHA PTx	FHA PTx	PTx, adenyl cyclase, tracheal cytotoxin & endotoxin	—
Manifestations	None	Runny nose, malaise, fever	<ul style="list-style-type: none"> <li>- Repetitive cough with whoops*, ending in vomiting</li> <li>- Leucocytosis</li> </ul>	<ul style="list-style-type: none"> <li>- Diminished paroxysmal cough</li> <li>- Complications, e.g. pneumonia, encephalopathy &amp; seizures.</li> </ul>
Isolation of the organism from nasopharyngeal secretions	—	Isolated in large numbers**	Rarely isolated	—
Antimicrobial treatment	—	Reduces the severity and duration	Has no effect on the progress of the disease	—

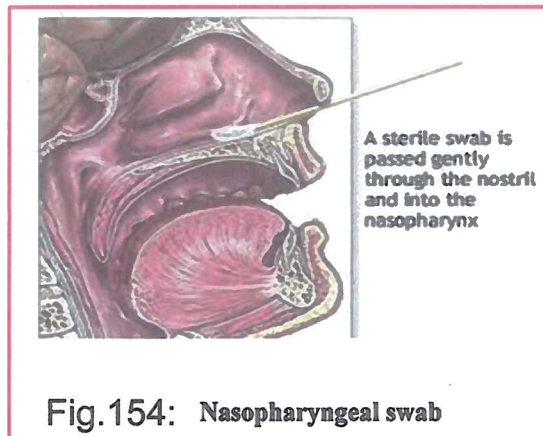
\* A characteristic sound produced by a rapid inspiratory gasp of air.

\*\*The patient is highly contagious during the catarrhal stage.

## Laboratory diagnosis

### A. Specimens

Nasopharyngeal secretions obtained by per-nasal swabs or cough plate. (Fig.154)



### B. Direct detection in nasopharyngeal secretions by:

1. The direct fluorescent-antibody (DFA) test
2. Nucleic acid detection by PCR

N.B.: Direct Gram stain is useless.

### C. Cultivation

- Culture is done on Bordet-Gengou medium or charcoal blood agar.
- Growth is slow and requires incubation for 5-7 days at 37°C.

### D. Identification

- Colony morphology: Colonies have mercury-droplet appearance.
- The organism is identified morphologically, serologically (by direct fluorescent antibody test or agglutination), or by PCR.

### E. Serology

It is of little help as antibodies are undetectable until the third week of illness.

## Treatment

- Antibiotic treatment is highly effective if given early. It helps eradication of the organism, reduces infectivity and decreases the risk of secondary complications.
- Erythromycin is the drug of choice. Trimethoprim-sulfamethoxazole is an alternative.

## Prevention

### A- Whooping cough vaccines:

#### 1. Killed whole cell vaccine:

- It is a part of the DPT vaccine (The P in DPT stands for pertussis cells).
- Unfortunately, about 20% of the children that receive the whole cell vaccine experience mild side effects.
- In a very small number of cases, severe or irreversible brain damage (encephalopathy) may occur especially if given after 6 years of age.

**2. Acellular vaccine:**

- It is a combination of pertussis toxoid (genetically inactivated PTx), filamentous haemagglutinin and other virulence factors.
- The acellular pertussis vaccine has fewer side effects than the whole cell vaccine and is currently recommended for use combined with diphtheria and tetanus toxoids as DTaP.
- Immunity wanes within 5-7 years. Accordingly, a booster is recommended after the age of 10 years.

**B- Chemoprophylaxis:** Erythromycin may be used for household contacts.

**MCQs:**

- 1- The virulence factors of *B. pertussis* include all the following **EXCEPT**:
  - a- Filamentous haemagglutinin
  - b- Pertussis toxin
  - c- Invasins
  - d- Tracheal cytotoxin
  - e- Adenyl cyclase toxin
- 2- Diagnosis of whooping cough **cannot** be done by:
  - a- Direct gram stain
  - b- Nucleic acid detection
  - c- Direct fluorescent antibody test
  - d- Culture on Bordet-Gengou medium
  - e- Culture on charcoal blood agar
- 3- DTaP is a vaccine developed from a combination of:
  - a- Diphtheria toxoid, tetanus toxoid and killed pertussis cells
  - b- Tetanus toxoid, killed diphtheria bacilli and pertussis toxoid
  - c- Diphtheria toxoid, killed *Cl. tetani* and pertussis toxoid
  - d- Diphtheria toxoid, tetanus toxoid and pertussis toxoid
  - e- Killed bacilli of *C. diphtheriae*, *Cl. tetani* and *B. pertussis*



## BRUCELLA

### ILOs:

**By the end of this chapter the student should be able to:**

- Describe and classify species of the *Brucella* genus
- Describe culture characteristics & growth requirements of *Brucella* species
- List the modes of transmission and recall the pathogenesis of *Brucella* species
- Describe laboratory diagnostic methods of brucellosis

### Characteristic features

1. Short Gram negative bacilli (coccobacilli) (Fig.155)
2. Aerobic
3. Facultative intracellular pathogens



Fig.155: *Brucella* (Gram stain)

Members of the genus *Brucella* are primarily pathogens of animals. Three major species can infect humans causing brucellosis. They are named after their animal hosts as follows: (Fig.156)

- 1- *B. abortus* infects cattle causing abortion.
- 2- *B. melitensis* infects sheep and goats.
- 3- *B. suis* infects pigs.

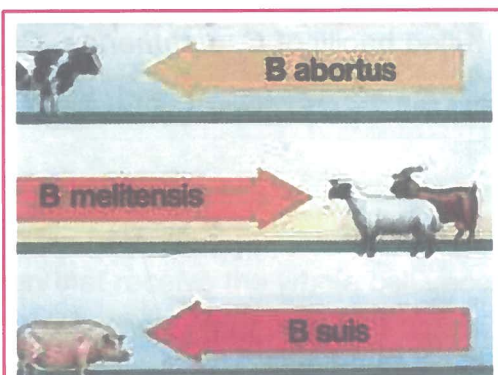


Fig.156: *Brucella* species

## Morphology (mentioned above)

## Culture

- *Brucella* can be grown on enriched media. (Fig.157)
- *B. abortus* requires 10% CO<sub>2</sub>.

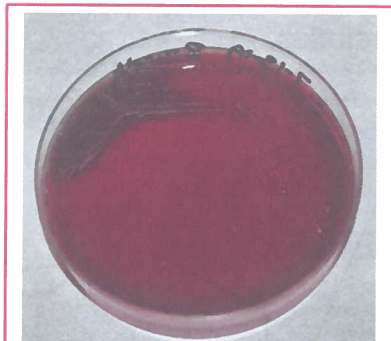


Fig.157: *Brucella* on blood agar

## Biochemical reactions

Different biochemical reactions help in identification of the genus *Brucella* and in distinguishing the different *Brucella* species.

## Antigenic structure

- The *Brucella* species have two distinguishing antigens (A and M) associated with the LPS of *Brucella* cell wall.
- The A antigen predominates in *B. abortus* whereas the M antigen predominates in *B. melitensis*.
- They can be detected by agglutination tests using specific antisera.

## Brucellosis (Undulant Fever or Malta Fever)

Brucellosis is a zoonotic disease

## Mode of transmission

Brucellae localize in the placenta and mammary glands of animals where they are shed in large numbers in uterine discharge and milk. The organisms are also shed in faeces and urine of infected animals.

Transmission occurs by: (Fig.158)

- 1- Ingestion of contaminated unpasteurized milk or milk products.
- 2- Direct contact through skin abrasions during handling of infected animals or their discharges. Therefore, brucellosis is an occupational disease affecting mostly butchers, farmers and veterinarians.
- 3- Inhalation of infected aerosol during handling of infected animals or *Brucella* cultures in the laboratories.

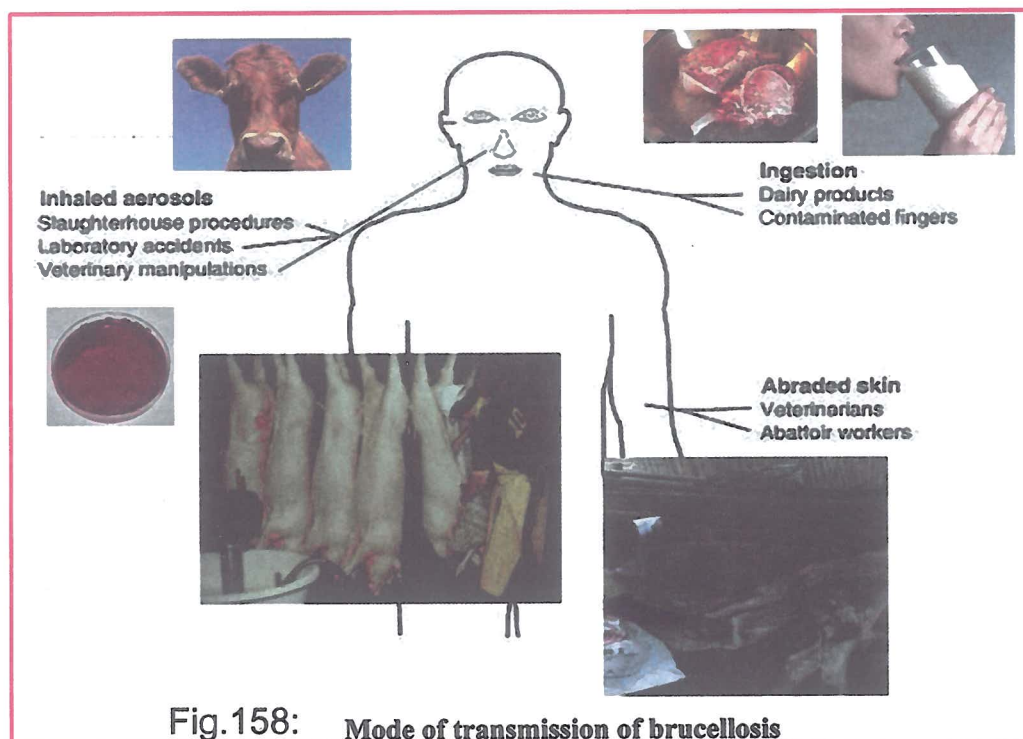


Fig.158: Mode of transmission of brucellosis

## Pathogenesis

- The endotoxin (LPS) is the major virulence factor.
- The organism passes from the site of entry to the regional lymph nodes, the thoracic duct, and thus into the blood (septicaemia).
- The organism localizes in the **reticulo-endothelial system**, namely lymph nodes, liver, spleen and bone marrow.
- Being a facultative intracellular parasite, it can survive and multiply within phagocytic cells. This results in **granuloma** formation (CMI response).

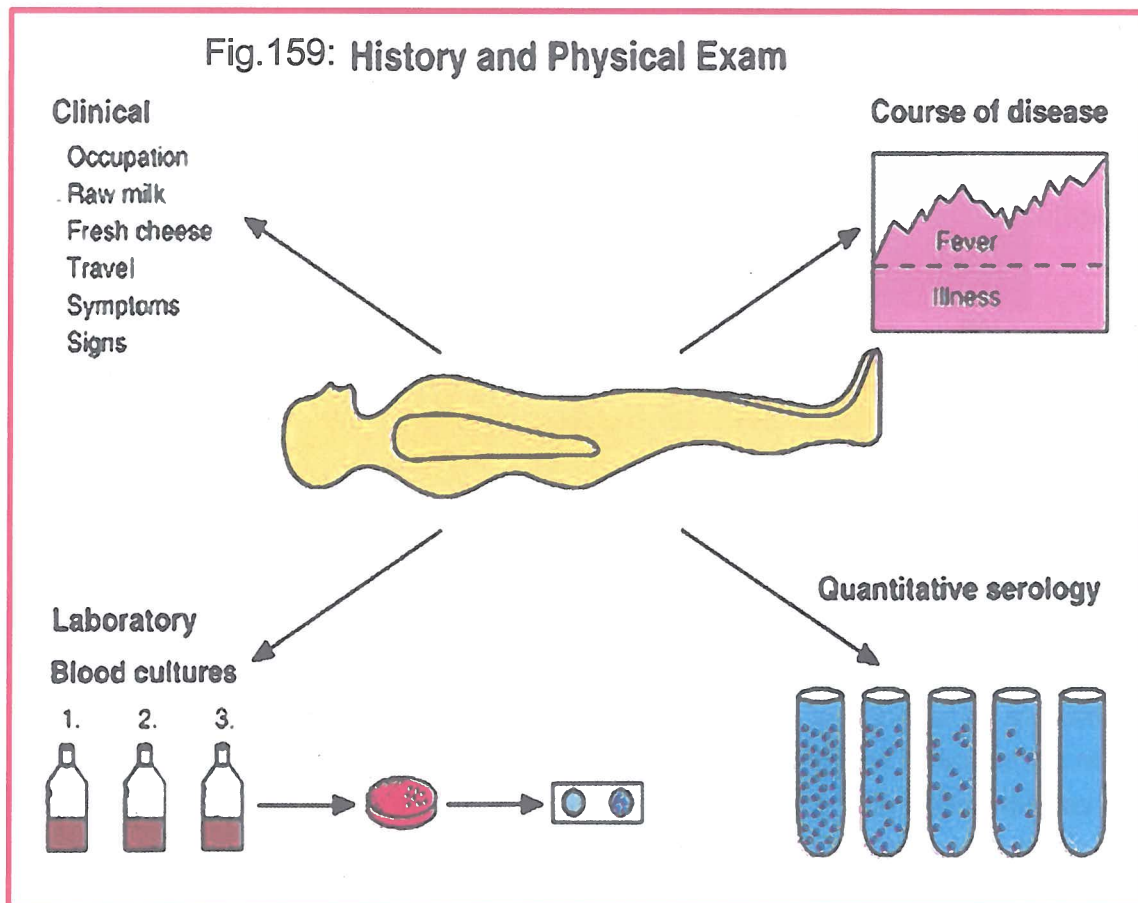
## Clinical manifestations

- Symptoms include fever, which is usually prolonged and intermittent (undulant), chills, weakness, malaise, body aches, sweating and headache.
- Enlarged lymph nodes, liver and spleen are frequently found.
- Complications include osteomyelitis, endocarditis and meningitis.
- Recovery usually occurs after a few weeks or months, but chronic stage (more than 1 year's illness) can develop.



## Laboratory diagnosis

Because the non-specific symptoms may not point to diagnosis of brucellosis, a detailed history is often crucial, including the patient's occupation and exposure to animals. (Fig.159)



### A. Specimens

- Blood or bone marrow during the acute illness.
- Other samples may be needed according to the affected sites.

### B. Cultivation

Isolation of the organism by the blood culture technique is rarely done because:

- There is a high risk to laboratory personnel to acquire infection by inhalation.
- The culture requires a long incubation (6-7 weeks).
- Being fastidious, the organism needs special growth requirements which are not always available.

**C. Serologic diagnosis: (Fig.160)**

It is the most frequently used diagnostic method:

**1. Standard tube agglutination test (STAT):**

STAT detects antibodies (IgM + IgG) to the three *Brucella* spp. A single titre of  $\geq 160$  or a fourfold rise in titre or greater is considered significant. False negative results may be due to:

- a- The **prozone** effect that is commonly encountered in cases of brucellosis, therefore wide range of serum dilutions (up to 1:5120) should be done.
- b- The presence of **incomplete or blocking antibodies**, which are produced in certain cases and obscure agglutination; therefore, Coomb's test should be done for negative results.

**2. ELISA for IgG or IgM.****D. Brucellin test**

It is a skin test similar to tuberculin test and is based on delayed type hypersensitivity. It is rarely used.

**Treatment**

Treatment of brucellosis requires combination of antibiotic therapy for a prolonged period (6 weeks) due to the intracellular residence of the organisms. Doxycyclines and rifampin are the combination of choice.

**Prevention**

- 1- Pasteurization of milk and its products.
- 2- Control of infections in animals, e.g. by using live attenuated vaccine for cattle.

**MCQs:**

- 1- Regarding *Brucella*, one of the following statements is correct:
  - a- *Brucella* species are transmitted primarily by tick bite.
  - b- The principal reservoirs of brucellae are small rodents.
  - c- Brucellae localize in the reticuloendothelial system and often cause granulomatous lesions.
  - d- Brucellae are obligate intracellular parasites that grow only in human cell culture.
  - e- Brucellae cannot grow on ordinary media.
  
- 2- The following methods can be used for the diagnosis of brucellosis EXCEPT:
  - a- Blood culture
  - b- Standard tube agglutination test (STAT) for total antibody (IgG + IgM)
  - c- Widal test
  - d- Brucellin test
  - e- ELISA for IgG or IgM
  
- 3- Regarding the standard tube agglutination test (STAT) for diagnosis of brucellosis, all the following statements are true EXCEPT:
  - a- A prozone effect is commonly encountered.
  - b- A wide range of serum dilutions should be done.
  - c- It detects antibodies to *Brucella abortus* only.
  - d- Blocking antibodies may give false negative results.
  - e- A titer of 160 or more is diagnostic for brucellosis.
  
- 4- Fever of unknown origin in a farmer who raises goats would most likely be caused by:
  - a- *S. aureus*
  - b- *Clostridium difficile*
  - c- *Brucella melitensis*
  - d- *Mycobacterium tuberculosis*
  - e- *Salmonella* Typhi



## LEGIONELLA

### ILOs:

By the end of this chapter the student should be able to:

- Identify the morphological characteristics of *Legionella pneumophila* and list areas of reservoir
- Describe the culture characteristics & growth requirements of *L. pneumophila*
- Demonstrate the pathogenesis and list diseases caused by *L. pneumophila*
- Outline laboratory diagnostic methods for infections due to *L. pneumophila*

The genus *Legionella* contains many species that normally live in water.

*L. pneumophila* is the major pathogenic species. It causes Legionnaire's disease (atypical pneumonia) and Pontiac fever (mild flu-like illness without pneumonia).

### *Legionella pneumophila*

#### Morphology

*L. pneumophila* is a motile, faintly stained Gram-negative bacillus.

#### Culture

*L. pneumophila* is an aerobic, highly fastidious organism. It can be grown on buffered charcoal yeast extract (BCYE) agar. (Fig.161)

#### Reservoir (Fig.162)

*L. pneumophila* is frequently found in water sources, e.g.:

1. water in cooling tanks and in air conditioners
2. within free-living amoebae in environmental water
3. within environmental biofilms.



Fig.161: *Legionella* on BCYE agar

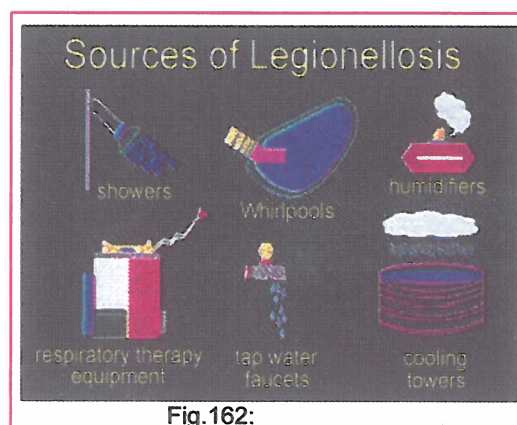


Fig.162:

## Mode of transmission

Inhalation of contaminated water aerosols. Outbreaks have been attributed to the presence of the organism in water taps, sinks, showers and air-conditioners.

N.B.: There is no person-to-person transmission.

## Pathogenesis

- Aside from endotoxin, no other virulence factors are known.
- The organism replicates intracellularly within alveolar macrophages (facultative intracellular).
- Accordingly, cell-mediated immunity determines the type of clinical presentation:
  - Immunosuppressed patients develop pneumonia (Legionnaire's disease).
  - Healthy individuals develop flu-like illness (Pontiac fever).

## Diagnosis

- Culture of respiratory secretions on BCYE agar.
- Detection of antigen in urine provides rapid diagnosis.
- Detection of rising antibody titre in patient's serum.

## Treatment

Azithromycin or erythromycin with rifampin for immunocompromised patients.

## Prevention

Routine decontamination of water tanks by high temperature and hyperchlorination.

### MCQs:

- 1- Legionnaire's disease is a form of:
  - a- Food poisoning
  - b- Sexually transmitted disease
  - c- Atypical bacterial pneumonia
  - d- Meningitis
  - e- Neonatal infection
- 2- Rapid diagnosis of Legionnaire's disease can be provided by antigen detection in:
  - a- Blood
  - b- Serum
  - c- Stools
  - d- Urine
  - e- CSF

## GRAM-NEGATIVE ANAEROBIC BACILLI

### ILOs:

**By the end of this chapter the student should be able to:**

- Recognize the morphology of *Bacteroides* and *Fusobacterium* species
- Recall the habitat of *Bacteroides* and *Fusobacterium* species
- List modes of transmission of *Bacteroides* and *Fusobacterium* species
- Outline infections caused by *Bacteroides* and *Fusobacterium* species

### BACTEROIDES

*Bacteroides* are the predominant organisms found in the human colon. *B. fragilis* is the commonest pathogenic species.

#### Morphology

Gram-negative capsulated bacilli.

#### Transmission

*Bacteroides* are transmitted via gut contents to the blood or peritoneum during abdominal surgery or following trauma (i.e. endogenous infection).

#### Virulence factors

The antiphagocytic capsule is the most important virulence factor.

#### Diseases

*B. fragilis* causes peritonitis (often mixed infection), abdominal abscess and bacteraemia which may occasionally lead to infection of the head and neck.

#### Diagnosis

The organism can be cultured anaerobically on blood agar, and is identified by biochemical reactions and gas chromatography.

#### Treatment

- Most strains are resistant to penicillin but sensitive to metronidazole, clindamycin and chloramphenicol (especially for brain abscess).
- Prophylactic antibiotics may be used for bowel surgery.



**FUSOBACTERIUM**

- They are large Gram-negative fusiform or cigar-shaped bacilli.
- They are part of normal microbial flora in the mouth, GIT and female genital tract.
- Most infections with these organisms are of **endogenous** origin.
- In association with spirochaetes, they cause fusospirochaetal disease in the oral cavity (Vincent's angina).

**MCQs:**

- 1- **Peritonitis caused by *B. fragilis* is acquired:**
  - a- Faeco-orally
  - b- By ingestion of contaminated food
  - c- As a complication of food poisoning
  - d- As an endogenous infection
  - e- By a puncture wound
- 2- **Which of the following is correct about *B. fragilis*:**
  - a- It is the most likely organism to cause gastroenteritis following antibiotic treatment.
  - b- It is sensitive to penicillin.
  - c- It is the predominant organism in the human gastrointestinal tract.
  - d- It may cause flaccid paralysis which may lead to respiratory arrest.
  - e- It is the most common organism causing urinary tract infection.
- 3- ***Fusobacterium* contributes to the pathogenesis of:**
  - a- Acne vulgaris
  - b- Necrotizing fasciitis
  - c- Bacterial vaginosis
  - d- Vincent's angina
  - e- Honeymoon cystitis

## MYCOBACTERIUM

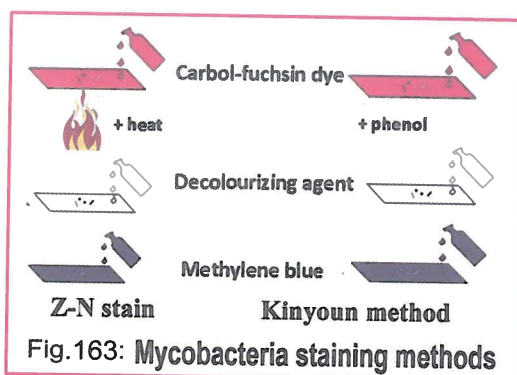
### ILOs:

By the end of this chapter the student should be able to:

- Describe and classify species of the *Mycobacterium* genus
- Describe morphology, culture characteristics and biochemical reactions of *Mycobacterium tuberculosis*
- Identify the distinguishing characteristics of the cell wall of *M. tuberculosis*
- Describe the transmission of *M. tuberculosis*
- Outline laboratory diagnostic methods for tuberculosis (infection and disease)
- Outline treatment measures of tuberculosis and recognize the resistance patterns to antimicrobial drugs
- Recognize the susceptibility of *M. tuberculosis* to physical and chemical agents and describe measures for prevention of tuberculosis
- Describe general features of non-tuberculous mycobacteria
- List important non-tuberculous mycobacteria and diseases they cause
- Describe the morphology of *Mycobacterium leprae*
- Compare and contrast tuberculoid leprosy with lepromatous leprosy
- Outline laboratory diagnostic methods for leprosy
- Recall treatment of leprosy

### Characters of the genus *Mycobacterium*

1. **Acid-fastness:** The cell wall of mycobacteria is rich in lipids (especially mycolic acid) that render them resistant to penetration by dyes used in Gram stain. However, they can be stained with special techniques such as Ziehl-Neelsen method or fluorochrome staining method. In these methods, the organisms firmly retain the dye used and resist decolourization even by acidic solutions; therefore, they are termed "acid-fast". (Fig.163)



2. **Slow rate of growth:** Mycobacteria have a longer generation time and, consequently, a slower rate of growth than ordinary bacteria. This growth rate varies among different species so that it permits their grouping into slow and rapid growers. Slow growers require more than 7 days to produce visible colonies on solid media, while rapid growers require less than 7 days.
3. **Obligate aerobe.**

### Members of the genus *Mycobacterium*

1. ***Mycobacterium tuberculosis* complex:** *M. tuberculosis* and three very closely related species (*M. bovis*, *M. africanum* and *M. microti*) compose what is known as the *M. tuberculosis* complex. Members of this complex are also called **tubercle bacilli**. They can cause tuberculosis in man and animals. Humans are the only reservoir for *M. tuberculosis* whereas both cows and humans are reservoirs for *M. bovis*.
2. **Nontuberculous mycobacteria (NTM),** e.g. *M. avium* and *M. fortuitum*.
3. ***Mycobacterium leprae*:** the causative organism of leprosy.
4. **Saprophytic mycobacteria:** rarely incriminated in human diseases.

### *Mycobacterium tuberculosis*

#### Morphology

- *M. tuberculosis* are slender acid-fast bacilli.
- They appear pink in a blue background when stained with the Ziehl-Neelsen or Kinyoun method. When stained with the auramine-rhodamine dye, AFB fluoresce orange yellow in a black background. (Fig.164,165)



Fig.164: *M. tuberculosis*  
(Auramine-rhodamine dye)

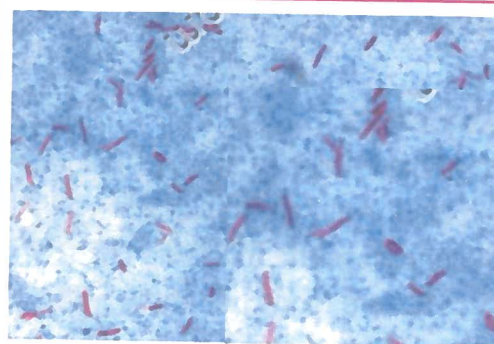


Fig.165: *Mycobacterium tuberculosis*  
(Z-N stain)



- Smears prepared from cultures (especially fluid media) may reveal bacilli arranged in a characteristic "**serpentine cords**"- like pattern. It is believed that this appearance is due to **cord factor** associated with virulent strains. (Fig.166)

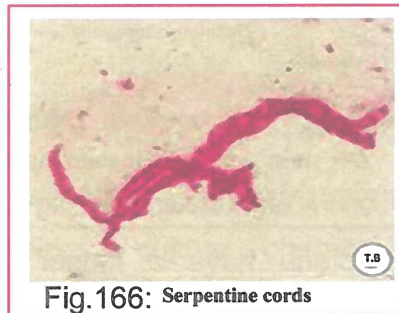


Fig.166: Serpentine cords

### Cultural characters

*M. tuberculosis* requires special media for growth:

1. **Lowenstein-Jensen (L-J) medium** which is an egg-based medium that is rendered selective by addition of malachite green dye. (Fig.167)

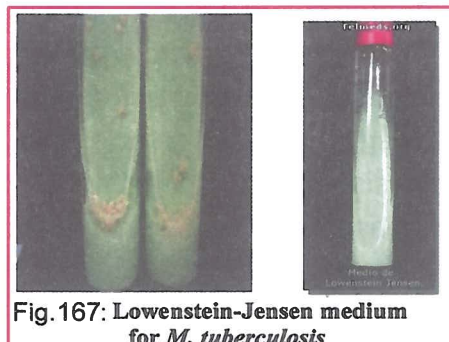


Fig.167: Lowenstein-Jensen medium for *M. tuberculosis*

2. **Middlebrook's medium** which is an enriched medium that can be rendered selective by addition of antibiotics. It may be fluid (broth) or solid (agar-based). Colonies appear after about 2-8 weeks of incubation at 37°C.

### Biochemical reactions

- Biochemical reactions are used to differentiate *M. tuberculosis* from other members of the genus.
- *M. tuberculosis* is positive for niacin production, nitrate reduction and production of heat-sensitive catalase.

### Cell wall structure

The cell wall of the tubercle bacilli is unique to the *Mycobacterium* species. It has a peptidoglycan layer which is similar to that of Gram-positive bacteria.

In addition, it comprises the following lipids:

1. **Mycolic acids:** are long chain fatty acids containing 60 to 90 carbons.
2. **Cord factor:** inhibits leucocyte migration and disrupts mitochondrial respiration.
3. **Mycobacterial sulfolipids:** inhibit phagolysosome formation.

The high concentration of lipids in the cell wall of *M. tuberculosis* has been associated with:

- a- Impermeability to stains and dyes.
- b- Resistance to drying.
- c- Resistance to many antibiotics.
- d- Resistance to killing by acidic and alkaline compounds.
- e- Resistance to osmotic lysis via complement deposition.
- f- Ability to survive inside macrophages and induce CMI response.

### Susceptibility to physical and chemical agents

1. Tubercle bacilli are killed by:
  - Heating at 55°C for 1 h, autoclaving, pasteurization and sunlight.
  - Intermediate level disinfectants such as ethyl and isopropyl alcohols and chlorine.
2. They survive for many weeks when dried in sputum smeared on clothing and in dust.
3. They are resistant to acids and alkalis, an important feature, which is used in cultivation procedures from contaminated specimens.

### Human Tuberculosis

Tuberculosis (TB) is a major cause of death in the world. The number of cases with TB has increased after the start of AIDS pandemic. Bacteria that cause the new outbreaks may be multidrug-resistant, which is considered a very serious situation.

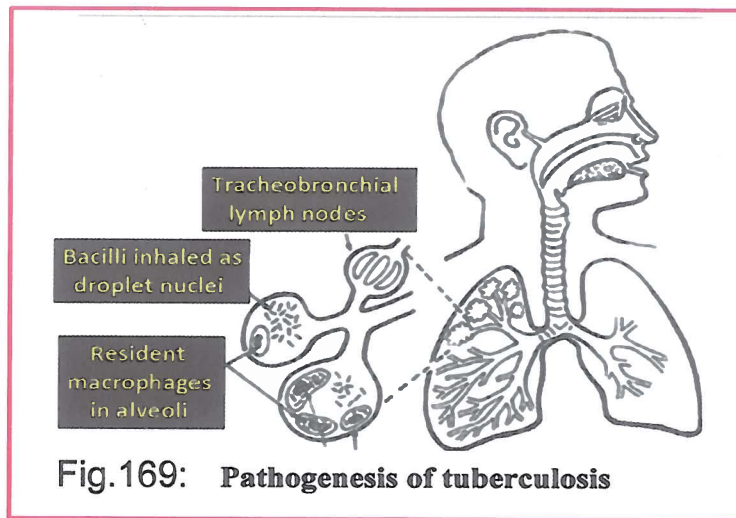
### Pathogenesis

- The organism may be transmitted by:
  - **Aerosol (airborne) infection** which is the commonest mode of infection due to inhalation of droplet nuclei carried by air from a patient with open pulmonary tuberculosis. (Fig.168)



Fig.168: Mode of transmission of tuberculosis (Airborne)

- Ingestion of milk contaminated with *M. bovis* which may cause intestinal infection.
- The droplet nuclei reach the terminal alveoli where they are engulfed by alveolar macrophages. The majority of these bacilli are destroyed or inhibited. (Fig.169)



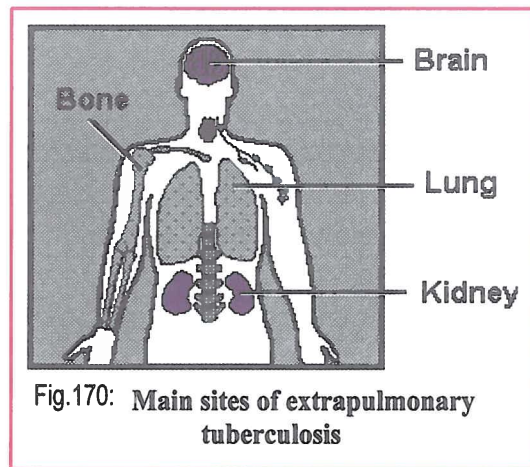
- The remaining bacilli are able to survive and multiply intracellularly by inhibiting fusion of phagosomes and lysosomes allowing intracellular survival (even if fusion occurs, the fatty nature of cell wall reduces killing effect). Its capacity to multiply both inside and outside cells, makes *M. tuberculosis* a facultative intracellular pathogen.
- Cell-mediated immune response, which is the main immune mechanism against mycobacteria, is initiated about 4-6 weeks after infection resulting in **granuloma (tubercle)** formation.
- Within the granuloma, *M. tuberculosis* can survive in small numbers in a relatively dormant state (**latent tuberculosis infection**). This situation is due to a balanced state of host-parasite relationship.

### Outcome of primary infection:

Primary tuberculosis may follow one of 2 courses:

- Latent tuberculosis infection:** The above mentioned events can all occur without development of symptoms. This occurs in about 90% of the infected people. Within 4-6 weeks after initial contact with the organism, these individuals become tuberculin and QuantiFERON positive (seroconversion).
- Tuberculosis disease:** In the remaining 10% of people, TB bacilli overcome the immune system and begin to multiply, resulting in the progression from TB infection to TB disease (Table 9). Symptoms of disease include general malaise, fatigue, night sweats and fever, along with persistent cough, and maybe bloody sputum. Tuberculosis may affect other systems, e.g. tuberculous meningitis, lymphadenitis, renal and intestinal tuberculosis. (Fig.170)





### Reactivation:

- Under certain conditions, e.g. immunosuppression, disturbance of the host-parasite balance may lead to **reactivation** of latent tuberculosis infection and development of tuberculosis disease.
- Reactivation usually occurs within 2 years after initial infection in about 5% of individuals with latent TB infection.
- The most common site of reactivation is the apex of the lung.

**Table (9): Latent tuberculosis infection versus disease**

Characters	Latent TB infection	Pulmonary TB disease
<i>M. tuberculosis</i> present	Yes	Yes
Tuberculin (or QuantiFERON) test	Positive	Positive
Sputum smears and cultures	Negative	Positive
Symptoms	No symptoms	Cough, fever, weight loss
Infectivity	Not infectious	Often infectious
Case definition	Not a case of TB	A case of TB

### Laboratory diagnosis of open pulmonary tuberculosis

The definitive laboratory diagnosis of tuberculosis disease depends on the detection and isolation of *M. tuberculosis* from clinical specimens.

**A. Specimens** include sputum, bronchoalveolar lavage...etc. Three early morning sputum specimens collected on consecutive days, from a deep productive cough, give the best results.

## B. Direct detection

1- **Smears** prepared directly from sputum are subjected to one of the following **acid-fast staining methods**:

- a. Ziehl-Neelsen (Z-N) or Kinyoun method, using the carbol-fuchsin dye. In Z-N method, heat is used to help penetration of carbol-fuchsin. In Kinyoun method, heat is replaced with phenol. Under the light microscope, AFB appear pink in a blue background.
- b. Fluorochrome staining with the auramine-rhodamine stain. Under the UV microscope, AFB fluoresce orange yellow in a black background. Compared to Z-N method, fluorochrome staining is:
  - More sensitive and faster; therefore, it is used for screening.
  - Less specific; therefore, positive results should be confirmed by Z-N stain.

A positive AFB sputum smear has the following advantages:

- Detection of AFB in stained smears may provide **early presumptive diagnosis** which allows rapid patient care. The result should be reported to the clinician within hours.
- Monitoring the infectivity of the patient and the response to anti-tuberculosis therapy.

However:

- A positive AFB smear can not differentiate between *M. tuberculosis* and other mycobacteria.
- A negative AFB smear does not exclude TB infection because organisms may be too few to be detected microscopically.

2- **Molecular methods**: PCR can be used for rapid (same day) detection of *M. tuberculosis* in sputum samples.

## C. Processing of sputum by liquefaction, decontamination and

**concentration**: This procedure is applied to sputum as well as other specimens contaminated with normal flora. It is done to prepare such specimens for culture and, sometimes, for smear examination and DNA detection. It enhances detection of the AFB because:

- Liquefaction of sputum releases trapped mycobacteria from tenacious specimens.
- The mycobacteria in the liquefied specimen can then be concentrated in a small sediment by centrifugation.
- Decontamination kills normal flora allowing obvious growth of the slowly growing mycobacteria.

**N-acetyl-L-cysteine - sodium hydroxide method:**

- It is the most commonly used method.
- It is based on the ability of NaOH to inhibit organisms other than mycobacteria.
- The mucolytic agent N-acetyl-L-cysteine (NALC) is combined with NaOH to help liquefaction of sputum.

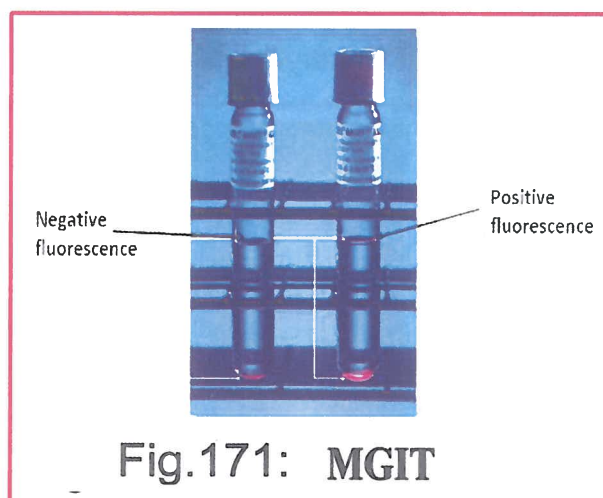
**D. Cultivation:** Definitive diagnosis of TB disease is achieved only by a positive culture. One of the following culture media can be inoculated by sediment of processed sputum and incubated in 5 to 10% CO<sub>2</sub> at 37°C:

**1- Conventional culture media (L-J and Middlebrook's agar media):**

- Non-pigmented, rough colonies appear after about 2-8 weeks of incubation.
- Colonies of *M. tuberculosis* are identified microscopically by Z-N stain and biochemically by nitrate reduction, niacin production and catalase tests.

**2- Fluid medium systems:** Middlebrook broth is used as the base for the following systems:

- Bactec AFB system:** Bactec medium contains radio-labelled palmitate as the sole carbon source. As *M. tuberculosis* multiplies, it breaks down the palmitate and liberates <sup>14</sup>C-labelled CO<sub>2</sub> that can be detected.
- Mycobacteria Growth Indicator Tube (MGIT):**(Fig.171)



- In this selective medium, bacterial growth will result in oxygen depletion.
- This can be detected by a fluorescence sensor that fluoresces upon exposure to UV light.

The major advantage of culture on these fluid systems is that they allow detection of growth in 4 to 14 days.

**E. Tuberculin and QuantiFERON tests** (see below).



## Laboratory diagnosis of extrapulmonary tuberculosis

Diagnosis of extrapulmonary TB such as tuberculous meningitis, lymphadenitis, renal and intestinal tuberculosis is performed as mentioned for pulmonary TB but by collecting the appropriate specimens. However, specimens collected from normally sterile sites, e.g. CSF, do not require decontamination.

## Laboratory diagnosis of latent tuberculosis

### A- Tuberculin Skin Test (TST)

#### Principle:

The tuberculin test is a skin test that detects delayed hypersensitivity response to previous exposure of the host to tubercle bacilli. Therefore, a positive TST indicates previous infection by the organism but not necessarily active disease.

#### Technique:

- Purified protein derivative (PPD), which is prepared from culture filtrate of the organism, is the antigen used in the tuberculin test.
- 0.1 ml of PPD containing 5 tuberculin units (TU) is injected intradermally in the skin of the anterior aspect of the forearm (Mantoux method).
- The result is read after 48-72 h by palpating for the presence of induration. The diameter of the induration (NOT the erythema) is measured in millimeters. (Fig.172)

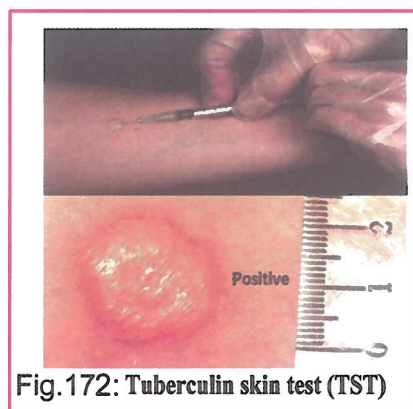


Fig.172: Tuberculin skin test (TST)




#### Underlying mechanism:

- As a result of previous exposure of the host to tubercle bacilli Th1 cells become sensitized.
- In positive reactors, the injected PPD stimulates the presensitized Th1 cells to secrete cytokines which recruit inflammatory cells particularly macrophages. The result is a **raised, indurated** area around the site of injection.
- No reaction is seen in people who have not been sensitized to TB.

**Interpretation of the tuberculin test:**

The reactions are categorized by different criteria (risk factors) depending on the circumstances of the patient. This is the so-called "5-10-15 millimeter system" (Table 10).

**Table (10):** Interpretation of the tuberculin skin test

 <b>An induration of 5 or more millimeters</b>	 <b>An induration of 10 or more millimeters</b>	 <b>An induration of 15 or more millimeters</b>
considered positive for: 1. People with past history of TB 2. Close contacts of infectious TB patients 3. People with HIV infection	considered positive for: 1. People in endemic areas where TB is common 2. People with certain medical conditions such as diabetes 3. Unvaccinated children younger than 4 years old	considered positive even in absence of any risk factor for TB

**False negative reactions:**

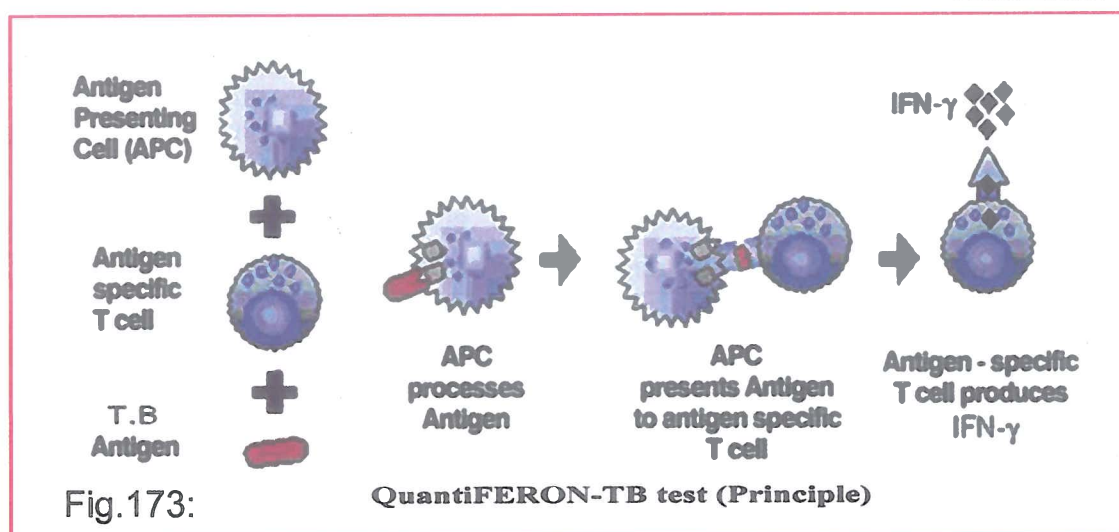
1. **Anergy:** The inability to react to TST may occur as a result of a weakened immune system, e.g. severe TB disease, HIV infection, or cancer.
2. **Recent TB infection:** After exposure, it takes 4 to 6 weeks for tuberculin test to become positive.

**False positive reactions:**

1. **Infection with nontuberculous mycobacteria (NTM)** due to cross-reaction with *M. tuberculosis* antigens.
2. **Vaccination with bacille Calmette-Guérin (BCG):** After BCG vaccination, tuberculin skin test remains positive for up to 5 years.

**B- QuantiFERON TB (IFN- $\gamma$  release assay)**

- Principle: Measurement of the amount of IFN- $\gamma$  released from the patient's sensitized T-lymphocytes after exposure to specific *M. tuberculosis* antigens in cell culture. (Fig.173)
- Compared to TST, this test is specific for diagnosing latent tuberculosis infection because the antigens used are not present in NTM or BCG vaccine strains.



### Treatment regimens of tuberculosis

- To be successful, the treatment should fulfill the following requirements:
  1. **Combination therapy:** Combination of 4 drugs or more is essential to reduce the drug toxicity and to prevent the emergence of drug resistant mutants.
  2. **Prolonged therapy:** this is because of:
    - the intracellular location of the organism
    - blocking of drug penetration by caseous material
    - the slow rate of growth of the organism
    - the presence of the persisters (metabolically inactive bacilli) within the lesion.
- Although therapy is given for at least 6 months, the patient's sputum becomes non-infectious within 2-3 weeks.
- Because of patients non-compliance, a regimen recommended by WHO known as directly observed therapy short course (DOTS) is thought to be effective for such patients.
- Anti-tuberculous drugs include:
  - **First-line drugs:** isoniazid (INH), rifampin, pyrazinamide, ethambutol and streptomycin.
  - **Second-line drugs:** fluoroquinolones, para-aminosalicylic acid, ethionamide, cycloserine, capreomycin, kanamycin, amikacin and rifabutin; these drugs are only used if resistance occurs during administration of first-line drugs.
- Resistance patterns:
  - Multidrug-resistance (MDR) means resistance of *M. tuberculosis* to both isoniazid and rifampin. It is a serious situation that presents difficulty in treatment.
  - Extensive (extreme) drug resistance (XDR) means MDR plus resistance to a fluoroquinolone and at least one additional drug.

*M. tuberculosis* isolates should be tested for drug resistance as early as possible in order to ensure appropriate treatment.



## Prevention and Control

### A. General measures

- Early case finding and effective treatment.
- Applying proper infection control measures in hospitals (e.g. use of N95 masks).
- Avoid overcrowding.
- Better housing and nutrition to improve host resistance.
- Pasteurization or boiling of milk.

**B. Treatment of latent infection:** To reduce the risk of progression to active tuberculosis, isoniazid (INH) is given for 6-9 months to:

- "Recent converters": Individuals who show recent conversion to a positive tuberculin or QuantiFERON test.
- Tuberculin or QuantiFERON positive individuals who are subject to immunosuppression.

### C. BCG vaccine:

- It is a live attenuated vaccine prepared from *M. bovis* and attenuated by repeated subcultures.
- It is routinely given to newborns intradermally in the left deltoid region.
- Although the protective efficacy of BCG vaccine in preventing TB infection is a subject of controversy, it proved to be effective in protection against childhood tuberculous meningitis and disseminated tuberculosis.
- Many attempts are being made to develop a better vaccine.

## Non-Tuberculous Mycobacteria (NTM)

Nontuberculous mycobacteria (NTM) include those *Mycobacterium* species that are not members of the *Mycobacterium tuberculosis* complex, hence the use of the terms "nontuberculous mycobacteria" or "mycobacteria other than tuberculosis" (MOTT). They are wide-spread in the environment and have been recovered from tap water, water of haemodialysis units and drinking-water distribution systems.

General features of NTM

- 1. Most NTM are morphologically indistinguishable from *M. tuberculosis*.
- 2. Most NTM grow well on media used to grow *M. tuberculosis*.
- 3. Growth characteristics (rate of growth and pigment production) classify NTM into 4 groups (I-IV). (Fig.174)

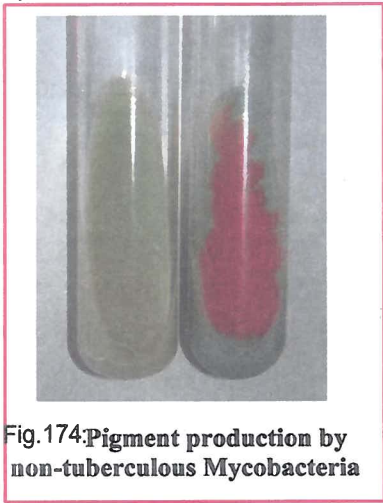


Fig.174:Pigment production by non-tuberculous Mycobacteria

- 4. NTM generally cause opportunistic infections (Table 11), particularly in immuno-compromised patients, e.g. AIDS patients.
- 5. Person-to-person transfer does not take place. Transmission is from environmental sources.
- 6. NTM are generally more resistant to antituberculous drugs. Combinations may have to be used (rifabutin, clarithromycin and azithromycin).
- 7. NTM show high resistance to a wide range of disinfectants.

Table (11): Examples of important NTM and the diseases they produce

Microorganism	Disease
<u>Slow growers</u> <i>M. avium-intracellulare</i> complex (MAC)	Pulmonary TB-like disease, particularly in AIDS patients
<i>M. kansasii</i>	Chronic lung disease resembling classical TB
<i>M. ulcerans</i>	Ulcerating infections of the skin (Buruli ulcer)
<u>Rapid growers</u> <i>M. fortuitum-chelonae</i> complex	Skin and soft tissue infections
<i>M. abscessus</i>	Chronic lung disease (which can disseminate to skin, bone and joints)

## *Mycobacterium leprae*

### Morphology

*M. leprae* closely resembles *M. tuberculosis* in size and shape. It occurs chiefly in bundles (globi) within the infected cells. *M. leprae* is less acid-fast than *M. tuberculosis*. Therefore, the use of the **modified Ziehl-Neelsen** method, in which the decolourizing agent is modified, is necessary to avoid overdecolourizing the AFB.

(Fig.175)

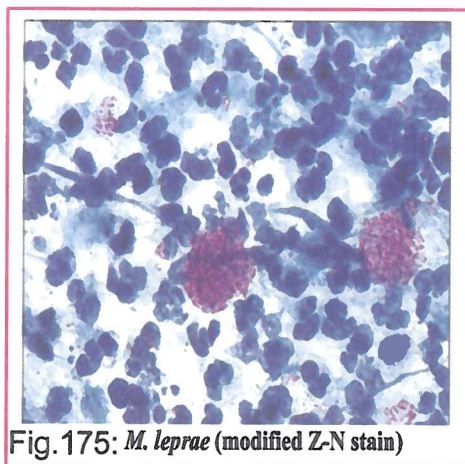


Fig.175: *M. leprae* (modified Z-N stain)

### Culture

- **In vitro:** *M. leprae* has not yet been successfully cultured *in vitro*. It is considered an obligate intracellular pathogen.

- **In vivo:**

1. **The nine-banded armadillo:** The nine-banded armadillo is a natural reservoir for *M. leprae*. This animal has become the main source of *M. leprae* for biochemical and immunological research including development of a vaccine.

(Fig.176)

2. **Mice:** Inoculation of the specimen into the hind foot-pads of mice is used to test the sensitivity of the bacilli to new drugs. (Fig.177)



Fig.176: *In-vivo* culture of *M. leprae*  
Nine-banded armadillo



Fig.177: *In-vivo* culture of *M. leprae*  
White mouse



## Leprosy

Leprosy (Hansen's disease) is a chronic infectious disease that has historically been more feared than any other infectious disease.

### Pathogenesis and clinical manifestations

- The exact mode of transmission is not certain. However, infection requires prolonged and close contact with patients.
- Infection may be transmitted by contact with skin lesions or aerosol inhalation.
- The clinical disease may develop years after initial contact with the organism; this is due to:
  - low infectivity of *M. leprae*
  - long generation time (slow rate of growth).
- Leprosy bacilli grow best at low temperature (optimally 30°C); therefore, the skin and superficial nerves are preferentially affected.
- The pathogenesis of leprosy appears to derive from:
  - 1) the ability of *M. leprae* to survive and replicate within macrophages, nerve cells and other host cells, and
  - 2) the consequent immune response to the organism.
- The disease presents in two basic forms: tuberculoid leprosy (TL) and lepromatous leprosy (LL), with many intermediate forms inbetween.

### Tuberculoid leprosy (TL) (Fig.178)

- Cell-mediated immune response mediated by Th1 cells predominates and forms granulomas, resulting in the destruction of most of the mycobacteria. So only few AFB remain in the tissues (paucibacillary leprosy).
- Lesions are few and mainly in the form of hypopigmented maculo-anaesthetic skin lesions.
- Lepromin skin test is positive.
- Although skin and peripheral nerves are damaged, TL progresses slowly, and carries a better prognosis than LL. In some patients it is self-limiting, but in others TL may progress across the spectrum towards LL.



Fig.178: Tuberculoid Leprosy

### Lepromatous leprosy (LL) (Fig.179)

- Cell-mediated immune response is depressed. Although humoral response mediated by Th2 cells is predominating, it is not protective as the organism is intracellular. The lesions usually contain large numbers of AFB (multibacillary leprosy).
- Lesions are mainly nodular and may form on the face. As the disease progresses, the nose may collapse giving the characteristic lionine facies. There is a marked sensory loss due to extensive nerve damage.
- Lepromin test is negative.
- LL is the more severe form and progresses rapidly.



Fig.179: Lepromatous Leprosy

Table (12): Comparison between tuberculoid leprosy and lepromatous leprosy

	<b>Tuberculoid leprosy</b>	<b>Lepromatous leprosy</b>
<b>Immune response</b>	Predominant CMI	Predominant AML
<b>T helper-type response</b>	Th1	Th2
<b>Lepromin test</b>	Positive	Negative
<b>Cytokine profile</b>	IL-2, IFN- $\gamma$ and TNF- $\beta$	IL-4, IL-5 and IL-10
<b>Number of AFB in tissues</b>	Few	Abundant
<b>Lesions</b>	Macular skin lesions	Nodular skin lesions
<b>Disease progress</b>	Slow	Aggressive
<b>Prognosis</b>	Good	Bad

### Laboratory diagnosis

Diagnosis is essentially a **clinical** one; laboratory diagnosis is done for confirmation:

**A. Specimen:** Slit skin smears, skin biopsy, or scrapings from the nasal mucosa.

**B. Direct detection:** Smears stained with modified Z-N method show intracellular AFB in bunches. A positive smear is sufficient for diagnosis.

**C. PCR assays.**

**D. The Lepromin skin test:** Lepromin is a heat-killed suspension of *M. leprae* prepared from infected armadillo tissue. It is positive in tuberculoid and negative in lepromatous leprosy. The test is of no diagnostic, but of prognostic value.

### Treatment

- Prolonged multidrug therapy is recommended to reduce development of resistance. The drugs used are rifampicin, clofazimine and dapsone. Among these, rifampicin is the most important anti-leprosy drug and is, therefore, included in the treatment of both forms of leprosy.
- In LL, triple therapy is recommended for a minimum of 2 years, whereas in TL a combination of dapsone and rifampicin is used for 6 months.
- Nerve damage and deformities are irreversible.

### MCQs:

- 1- *M. tuberculosis* can be isolated by culture on:
  - a- Blood agar
  - b- Loeffler's serum
  - c- Modified Thayer-Martin
  - d- Thiosulfate citrate bile salt sucrose (TCBS) agar
  - e- Lowenstein-Jensen medium
- 2- As regards the diagnosis of tuberculosis, the following are correct **EXCEPT**:
  - a- Three morning sputum samples should be examined.
  - b- Tuberculin test may give false positive and false negative results.
  - c- Detection of acid-fast bacilli in smears provides early presumptive diagnosis.
  - d- Liquefaction of tenacious samples is required before culture.
  - e- Growth on Lowenstein-Jensen medium appears after 48-72 hours.



- 3- **Regarding tuberculin test:**
- a- It depends on immediate hypersensitivity.
  - b- It depends on presence of sensitized Th2 cells.
  - c- A wheal and flare is considered positive.
  - d- People vaccinated with BCG give a negative result.
  - e- It is read after 48-72 hours.
- 4- **Treatment of tuberculous disease:**
- a- Is initiated with a single first line chemotherapeutic agent
  - b- Usually requires prolonged bed rest and surgery
  - c- Is continued until tuberculin test reverts to negative
  - d- Always results in negative sputum cultures and clinical cure within 3 weeks
  - e- Always necessitates the use of combined, prolonged drug therapy
- 5- **BCG vaccine, used for the prevention of tuberculosis is:**
- a- Heat killed vaccine
  - b- Formalin inactivated vaccine
  - c- Living attenuated vaccine
  - d- Recombinant vaccine
  - e- Bacterial toxoid prepared from *M. tuberculosis*
- 6- **Which of the following organisms cannot be grown on artificial media?**
- a- *S. pyogenes*
  - b- *S. epidermidis*
  - c- *K. pneumoniae*
  - d- *Salmonella Typhi*
  - e- *M. leprae*
- 7- **Tuberculoid leprosy has the following features EXCEPT:**
- a- Lepromin test is positive.
  - b- The course of the disease is benign.
  - c- Cell-mediated immunity is intact.
  - d- Acid fast bacilli are few in tissue smears.
  - e- Th2-type response is dominant.

## SPIROCHAETES

### ILOs:

**By the end of this chapter the student should be able to:**

- Define the general features of spirochetes
- Identify the morphological characteristics and culture of *T. pallidum*
- List the modes of transmission of *T. pallidum* and recognize the difference between venereal and congenital syphilis
- Define the different stages of untreated syphilis and outline their diagnosis
- Differentiate between treponemal and non-treponemal tests in the diagnosis of syphilis
- List the treatment measures of syphilis
- Identify the morphological characteristics and culture of *Borrelia*
- Compare and contrast types of relapsing fever, including their causes and vectors
- Describe pathogenesis and diagnosis of relapsing fever
- Review Lyme disease, its vector, causative agent and clinical findings
- Describe morphology and cultural characteristics of *L. interrogans*
- Describe pathogenesis and diagnosis of zoonotic leptospirosis
- Describe pathogenesis and diagnosis of fusospirochaetal disease

### Characteristic features

- Spirochaetes are slender, flexible, spiral rods. (Fig.180)
- They have a characteristic **corkscrew motility** due to the presence of axial filaments (endoflagella).
- They have a Gram-negative cell wall structure.
- Spirochaetes include anaerobic, microaerophilic and aerobic species.

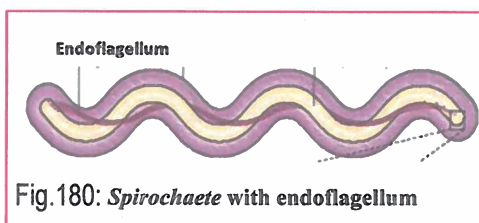


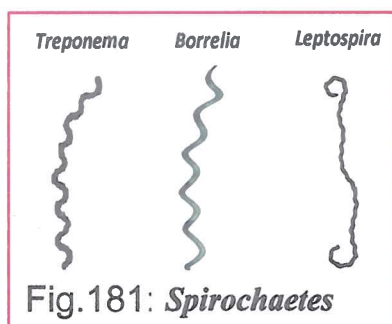
Fig.180: *Spirochaete* with endoflagellum

Three genera of spirochaetes cause human infections: (Fig.181)

1. *Treponema*

2. *Borrelia*

3. *Leptospira*



## *Treponema*

The most important species is *T. pallidum*, which causes syphilis (a sexually transmitted disease). *T. pallidum* is a human parasite. It has no animal or environmental reservoirs (because of its rapid death outside the host).

### Morphology

- *T. pallidum* has regular coils with pointed ends.
- The organism is very thin (1/5 the diameter of *E. coli*). It can only be seen by:
  - dark-field microscopy: in wet unstained preparations (Fig.182)
  - direct immunofluorescence

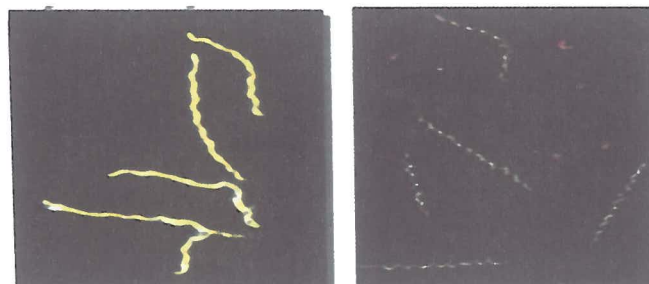


Fig.182: *T. pallidum* (dark field microscopy)

- light microscopy after impregnation (thickening) by silver staining (e.g., Fontana stain). (Fig.183)



Fig.183: *T. pallidum* (Fontana stain)

### Culture

- *In vitro*: *T. pallidum* cannot be grown on artificial culture media.
- *In vivo*: It can be maintained in the laboratory by inoculation of positive specimens into rabbit testicles.



## Syphilis

### Mode of transmission

1. Sexually: leading to venereal syphilis.
2. Transplacentally: leading to congenital syphilis.
3. **Fresh** blood transfusion: *T. pallidum* is not transmitted by stored blood because it dies when stored at 4°C within 3-5 days.

### Clinical forms

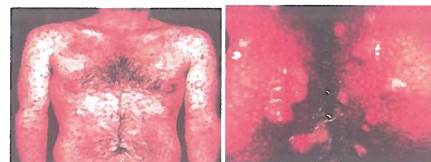
Classically, untreated syphilis occurs in the stages described in table (13):

**Table (13):** Stages of syphilis

Stage	Clinical manifestations	Diagnosis
Primary (2-6 weeks after exposure) (Fig.184)	<ul style="list-style-type: none"> <li>- Hard painless genital or oral ulcer (<b>chancre</b>)</li> <li>- Contagious (infectious)</li> <li>- Heals spontaneously (within 3-6 months)</li> </ul>	<ul style="list-style-type: none"> <li>- Direct detection of the organism</li> <li>- Serologic tests: reactive only late</li> </ul>
Secondary (1-3 months later) (Fig.185)	<ul style="list-style-type: none"> <li>- Four cardinal features:               <ol style="list-style-type: none"> <li>1. Generalized maculopapular skin rash</li> <li>2. Mucous patches in mouth</li> <li>3. Chondyloma lata around the genitals or anus</li> <li>4. Generalized lymphadenopathy</li> </ol> </li> <li>- Highly infectious</li> <li>- Spontaneous healing (within 3 months)</li> </ul>	<ul style="list-style-type: none"> <li>- Serologic tests</li> <li>- Direct detection of the organism</li> </ul>
Latent (may last for years)	- None	- Serologic tests only
Tertiary (years later) 30% of untreated cases (Fig.186)	<ul style="list-style-type: none"> <li>- Gumma formation (in skin and bones)</li> <li>- Cardiovascular syphilis</li> <li>- Neurosyphilis</li> </ul>	- Serologic tests only



**Fig.184:**Primary syphilis: Chancre



**Fig.185:** Mucous patches      Condyloma lata  
Secondary syphilis



**Fig.186:**Tertiary syphilis (Gumma formation)

### Congenital Syphilis:

*In utero* infections can lead to:

- abortion or stillbirth
- congenital abnormalities, which may be obvious at birth
- silent infection, in which manifestations may not be apparent until about 2 years of age (facial and tooth deformities).

### Laboratory diagnosis

1. **Direct detection** of the organism from chancre, mucous patches or chondyloma lata by:

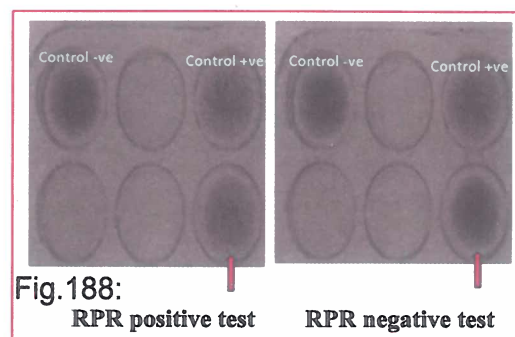
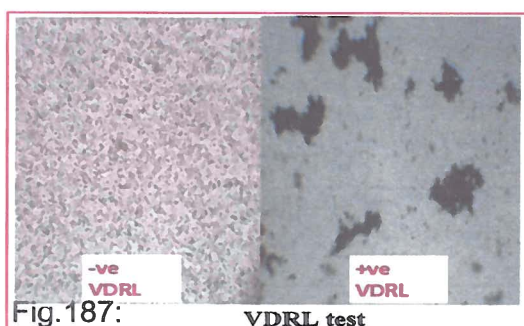
- Dark-field microscopy of wet unstained preparation, which detects the characteristic motility.
- Direct immunofluorescence: after staining with fluorescein-labelled anti-treponemal antibodies. This method is highly sensitive and specific.

2. **Serologic tests of syphilis:** They include:

- a- **Non-treponemal tests** which are non-specific tests used for screening.
- b- **Treponemal tests** which are specific tests used for confirmation.

### Non-treponemal tests

- Antibody detected:
  - These tests detect non-treponemal antibodies known as **reagin** (heterophil antibodies). These antibodies are produced in response to lipoidal material released from damaged host cells.
- Antigen used:
  - The antigen used is called **cardiolipin**, which is an extract from beef heart muscle with added lipids.
- Tests:
  - Non-treponemal tests include the following flocculation tests:
    - The venereal disease research laboratory (**VDRL**) test in which the flocculation is seen by microscopic examination. (Fig.187)
    - The rapid plasma reagin (**RPR**) test in which the flocculation is seen with the naked eye. (Fig.188)



- Applications:
  1. **Screening:** As these tests are inexpensive, rapid and simple, they are used for screening.  
However, being non-specific, they may give positive results in other conditions like autoimmune diseases, pregnancy, leprosy, viral infections and immunization. Therefore, positive results should be confirmed by one of the specific treponemal tests.
  2. **Follow up:** Since reactivity declines within 6-18 months after successful treatment, these tests are used for follow up.

### Treponemal tests

- Antibody detected:
  - These tests detect specific treponemal antibodies.
- Antigen used:
  - The antigen used is *T. pallidum* antigen.
- Tests:
 

Treponemal tests include the following tests:

  - Fluorescent treponemal antibody (FTA) test. (Fig.189)
  - *T. pallidum* haemagglutination assay (TPHA).(Fig.190)
  - Enzyme immune-assay (EIA) and Western blot.
- Applications:
  - **Confirmation:** Being specific, they are used in confirming or ruling out reactive non-treponemal test results.

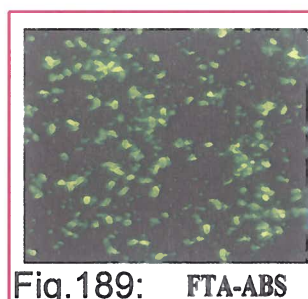


Fig.189: FTA-ABS

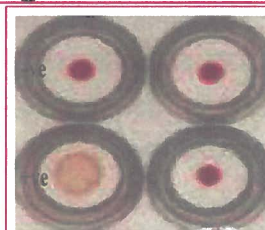


Fig.190: TPHA

N.B: - Treponemal tests are not used for screening, as they are more expensive and more difficult to perform than non-specific tests.

- They cannot be used to determine the response to treatment, as they remain reactive for life even after effective treatment.

**Table (14):** Comparison between non-treponemal and treponemal tests

	Non-treponemal tests	Treponemal tests
Specificity	non-specific	specific
Antibody detected	reagin Ab	anti-treponemal Ab
Antigen	Cardiolipin	treponemal antigens
Tests	VDRL, RPR	FTA, TPHA, EIA
Cost & performance	inexpensive, rapid, simple	expensive, time consuming, difficult to perform
Uses	screening & follow up of treatment	confirmation
Reactivity	6-18 months after treatment	for life



## Diagnosis of congenital syphilis

It is done by detection of treponemal IgM antibodies in newborn's serum by EIA.

## Treatment

- Penicillin is the drug of choice for treatment of syphilis. No resistance to penicillin has been recorded.
- Tetracycline, erythromycin and chloramphenicol can be used as alternative antibiotics for patients allergic to penicillin.
- Only penicillin or chloramphenicol can be used for patients with neurosyphilis.
- Syphilitic pregnant mothers should be adequately treated to prevent congenital syphilis.

## *Borrelia*

- *Borrelia* can be pathogenic for humans, domestic animals and rodents.
- In humans they cause relapsing fever and Lyme disease.
- Ticks transmit all known species of *Borrelia* except *B. recurrentis* which is transmitted by the human body louse.

## Morphology

- Borreliae are highly motile spirochaetes with irregular loose coils.
- They are best visualized with Giemsa stain. Some are Gram negative.

## Cultural characteristics

- Borreliae are microaerophilic, slowly growing spirochaetes.
- They grow on highly enriched media containing serum and tissue extract.

## Relapsing Fever

Relapsing fever is a febrile, septicaemic disease in which several relapses may occur.

## Transmission

There are two forms of relapsing fever:

1. **Tick-borne (endemic) relapsing fever**, which is caused by a variety of *Borrelia* species. The most important species is *B. hermsii*. Rodents and small animals are the main reservoir from which they are transmitted to man (zoonotic disease).
2. **Louse-borne (epidemic) relapsing fever**, which is caused by *B. recurrentis*. Man is the only reservoir. It is transmitted from man to man by human body louse (not a zoonotic disease).

## Clinical Manifestations

- Symptoms appear after an incubation period of 3 to 10 days.
- These symptoms include sudden onset of fever, severe headache and general malaise.
- Fever lasts for 3 to 5 days, followed by an afebrile period which lasts about a week before a second attack of fever starts. These relapses may be repeated 3 to 7 times.
- Relapses occur as a result of antigenic variations in the causative *Borrelia* spp. As antibodies develop against antigens of the existing organism, new antigenic variants emerge and produce relapses of the illness.

## Laboratory diagnosis

Diagnosis is usually made during the febrile stage, where abundant spirochaetes are present in the blood. This is done by detecting loosely coiled spirochaetes in blood films stained by Giemsa stain. (Fig.191)

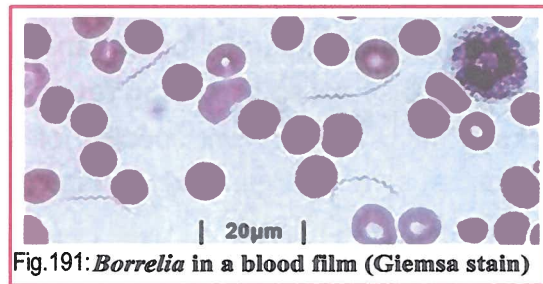


Fig.191: *Borrelia* in a blood film (Giemsa stain)

## Treatment

Tetracycline may be beneficial early in the illness and may prevent relapses.

## Lyme Disease

- The disease is caused by *B. burgdorferi*, which is transmitted by ticks bite.
- Although caused by different spirochaetes, the general similarities in the progression (stages) of Lyme disease and syphilis are striking. The clinical findings have been divided into 3 stages:
  - Stage 1: A characteristic spreading circular red lesion with a clear center (erythema migrans) occurs at the site of tick bite. (Fig.192)



Fig.192: Lyme disease (erythema migrans) caused by *Borrelia burgdorferi*

- Stage 2: The organism disseminates via blood resulting in muscle and joint pain (arthralgia), secondary skin lesions and lymphadenopathy.
- Latent stage.
- Stage 3: This stage is characterized by arthritis (specially large joints), CNS and cardiac dysfunction.
- The disease can be diagnosed serologically by ELISA, to be confirmed by Western blot.

## *Leptospira*

Leptospirae have a worldwide distribution. They may be free-living or may live in association with human or animal hosts. *Leptospira interrogans* is the causative agent of leptospirosis (Weil's disease), which is a zoonotic disease.

### Morphology

- Leptospirae are very thin and tightly coiled motile rods with hooked ends.
- They can be seen by dark-field microscopy.

### Culture

- *Leptospira interrogans* is an obligate aerobe.
- It can be grown on serum-containing media.
- Growth usually occurs within 1-2 weeks.

## Leptospirosis

### (Weil's Disease; Infectious Jaundice)

Rodents, dogs, swine and cattle act as reservoirs for the leptospirae. Infected animals excrete large numbers of the organism in urine resulting in contamination of water and soil where leptospirae remain viable for several weeks.

### Mode of transmission

- Contact with contaminated water where the organism enters through small skin abrasions. This may occur:
  - as an occupational hazard, affecting mainly farmers, sewage workers and miners.
  - during practicing water sports, e.g., swimming. (Fig.193)



Fig.193: Leptospirosis (infectious jaundice)



- Consumption of contaminated food or drink where the organism penetrates the mucous membrane.

### Clinical manifestations

The incubation period is usually 7 to 14 days.

Leptospirosis is typically a biphasic illness with a quiescent period inbetween:

1. **The first phase** is characterized by a febrile influenza-like illness due to blood invasion (septicaemia).
2. **The second phase** is characterized by dysfunction of liver (jaundice), kidney (uraemia) and CNS (aseptic meningitis) due to invasion of these organs by leptospirae from the blood stream. (Fig.194)



Fig.194: Leptospirosis (infectious jaundice)

### Laboratory diagnosis

Diagnosis is based on:

1. History of possible exposure, together with suggestive clinical signs.
2. Marked rise in specific IgM (highly sensitive).
3. Occasional isolation of the organism from blood, urine or CSF.

### Treatment

Penicillin or doxycycline are the drugs of choice.

## Fusospirochaetal Disease

Under certain circumstances, particularly injury to oral mucous membranes, nutritional deficiency or concomitant infection (e.g. with HSV), the normal spirochaetes of the mouth, together with anaerobic fusiform bacilli (fusobacteria) find suitable conditions for multiplication. They increase in numbers causing:

1. **Trench mouth:** A condition of acute necrotizing ulcerative gingivitis (ANUG).
2. **Vincent's angina:** Fusospirochaetal infection of the pharynx with pseudomembrane formation, similar to diphtheria and follicular tonsillitis. (Fig.195)



Fig.195: Vincent's angina

### Laboratory diagnosis

Gram-stained smear from the pseudomembrane shows large number of Gram-negative fusiform bacilli and spirochaetes in association with pus cells and other commensals. (Fig.196)

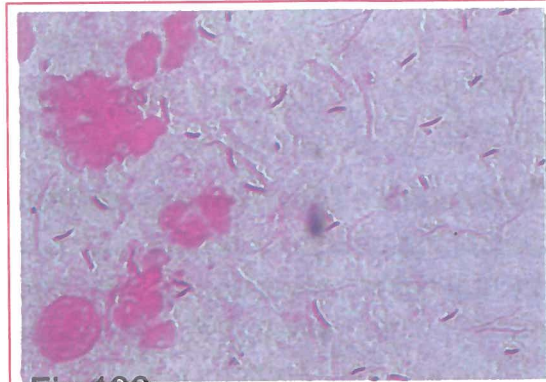


Fig.196: Vincent's angina (Gram stain)

#### MCQs:

- 1- All the following statements about primary syphilis are correct **EXCEPT**:
  - a- It manifests as a painless ulcer.
  - b- The characteristic lesion contains no organisms.
  - c- It can be diagnosed serologically during the late stage.
  - d- It is caused by *Treponema pallidum*.
  - e- It can be treated by penicillin.
- 2- Direct detection of *T. pallidum* cannot be done from:
  - a- Chancre
  - b- Mucous patches
  - c- Chondyloma lata
  - d- Gumma of internal organs
  - e- Any of the above
- 3- One of the following is a non-treponemal test:
  - a- The fluorescent treponemal antibody absorption
  - b- *T. pallidum* haemagglutination assay
  - c- The rapid plasma reagin test
  - d- The Western blot
  - e- The EIA
- 4- *Borrelia recurrentis*:
  - a- Is the causative organism of epidemic typhus
  - b- Can be detected in blood films after staining with Giemsa stain
  - c- Can be grown on simple media
  - d- Is an antigenically stable organism
  - e- Is transmitted among individuals by ticks

- 5- Weil's disease (infectious jaundice) is caused by:
- a- *Rickettsia typhi*
  - b- *Borrelia recurrentis*
  - c- *Coxiella burnetii*
  - d- *Leptospira interrogans*
  - e- *Borrelia burgdorferi*
- 6- Regarding Vincent' angina all the following statements are correct EXCEPT:
- a- It is an infection of the pharynx.
  - b- It is caused by oral spirochaetes and fusiform bacilli.
  - c- It is characterized by the formation of a pseudomembrane.
  - d- It may occur following viral infections e.g. HSV.
  - e- The causative organism(s) cannot be stained by Gram stain.



## MYCOPLASMA

### ILOs:

By the end of this chapter the student should be able to:

- List the main characteristics of *Mycoplasma*
- Recognize *Mycoplasma* species, diseases and modes of transmission
- Outline measures for laboratory diagnosis of *Mycoplasma* infection

### Characteristic features

1. Mycoplasmas are **bacteria without cell wall**. The lack of a cell wall renders these organisms:
  - a- Resistant to antibiotics which inhibit cell wall synthesis (e.g., beta-lactam antibiotics).
  - b- Unstainable by the Gram stain.
  - c- Variable in shape (pleomorphic). (Fig.197)

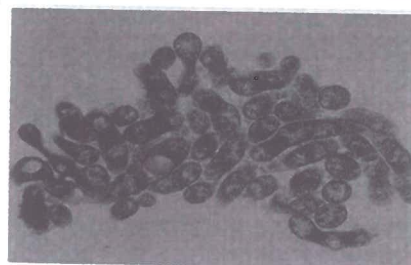


Fig.197: Electron microscopical appearance of *Mycoplasma* showing pleomorphism

2. Their contents are enveloped by a cell membrane.
3. They are the only bacteria that contain sterol (in the form of cholesterol) in the cell membrane.
4. They are the smallest bacteria that can be grown on cell-free media. They require serum-enriched medium containing cholesterol. After several days of incubation, mycoplasmas produce minute colonies which have a characteristic "fried-egg" appearance that can only be visualized microscopically. (Fig.198)

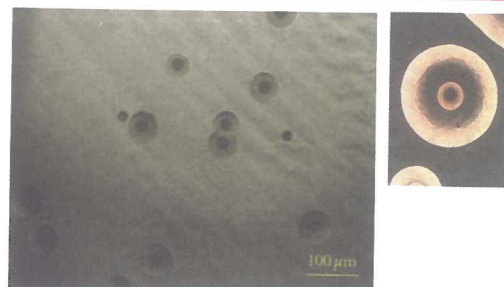


Fig.198: *Mycoplasma* colonies with "fried-egg" appearance

**Table (15):** *Mycoplasma* species, diseases and modes of transmission in humans

Species	Disease	Transmission
<i>Mycoplasma pneumoniae</i>	1. Atypical pneumonia (20% of pneumonia cases) 2. Pharyngitis, bronchitis & otitis media	Inhalation of respiratory droplets
<b>Genital mycoplasmas</b> 1. <i>M. hominis</i> 2. <i>M. genitalium</i> 3. <i>Ureaplasma urealyticum</i>	1. Nongonococcal urethritis 2. Pelvic inflammatory disease (PID) 3. Post-abortion or postpartum fever	Sexual

### Laboratory diagnosis

- Diagnosis of *Mycoplasma* infection is based on **serology** because:
  - Direct microscopical detection is of limited value.
  - Cultivation is difficult and requires prolonged incubation (up to 3 weeks in case of *M. pneumoniae*).
  - PCR, although rapid and highly sensitive, does not distinguish between colonization and infection.
- The serological tests used are:
  - 1- Specific tests:  
Detection of IgM or a fourfold rise in IgG antibody titre by EIA is diagnostic.
  - 2- Nonspecific test:  
Detection of **cold agglutinins**, which are IgM antibodies that agglutinate red cells at 4°C but not at 37°C. A titre of  $\geq 1:128$  of cold agglutinins is indicative of recent infection.  
However:
    - Positive results may occur in some viral infections, malaria, haemolytic anaemia and liver disorders; most of these diseases have symptoms that easily distinguish them from those of primary atypical pneumonia.
    - Negative results may occur in about 50% of cases of *M. pneumoniae* infections.

N.B.: Some patients develop very high titres of cold agglutinins. This may result in:

- Ischaemia and even necrosis of hands and feet because of *in vivo* clumping of red blood cells when the patient is exposed to cold temperature.
- Mild haemolytic anaemia due to *in vivo* destruction of red blood cells throughout the body.

### Treatment

Erythromycin and tetracycline are the drugs of choice for all mycoplasmas.

## MCQs:

- 1- ***Mycoplasma* species are characterized by all of the following EXCEPT:**
  - a- *M. pneumoniae* is one of the causes of atypical pneumonia.
  - b- Some species are transmitted sexually.
  - c- They are best treated with penicillin.
  - d- They are unstainable by Gram stain but readily stainable by Giemsa stain.
  - e- They are the only organism that contains sterol in the cell membrane.
- 2- **The lack of cell wall in *Mycoplasma* renders the organism:**
  - a- Soluble in bile
  - b- Unstainable by Giemsa stain
  - c- Variable in shape
  - d- Unable to grow in cell-free culture media
  - e- Resistant to quinolones
- 3- ***Mycoplasma* differs from *Chlamydia* in that they are:**
  - a- Susceptible to penicillin
  - b- Able to grow on artificial cell-free media
  - c- Able to cause non-gonococcal urethritis
  - d- Able to stain well with Gram-stain
  - e- Able to cause disease in humans



## CHLAMYDIA

### ILOs:

By the end of this chapter the student should be able to:

- List the main characteristics of *Chlamydia*
- Describe the life cycle of *Chlamydia*, including the two morphological forms.
- List diseases associated with the various *Chlamydia trachomatis* serovars, with *Chlamydophila psittaci*, and with *Chlamydophila pneumoniae* and their pathogenesis
- Outline diagnostic measures for infections caused by *Chlamydia trachomatis*
- List measures for treatment and prevention of *Chlamydia trachomatis*

### Characteristic features

1. *Chlamydia* is a genus of very small obligate intracellular bacteria. They lack the ability to produce sufficient energy to grow independently and, therefore, can grow only inside host cells.
2. In the lab, they can be grown on tissue culture.
3. Their cell wall structure resembles that of Gram-negative bacteria but lacks typical peptidoglycan layer.
4. Gram stain is not useful; but the organism can readily be stained by Giemsa stain.
5. Chlamydiae have a unique biphasic life cycle.

N.B.: Although chlamydiae are similar to viruses in being small obligate intracellular organisms, they are considered bacteria because of the differences mentioned in table (15).

### Life cycle (Fig.199)

- There are two morphological forms of *Chlamydia*:
  - **Elementary bodies (EBs)**: They are small, extracellular, metabolically inert particles. They represent the infectious form.
  - **Reticulate bodies (RBs)**: They are larger, intracellular, metabolically active particles. They represent the replicating form.
- The EBs bind to specific host cell receptors by protein adhesins and enter the cells by endocytosis.

- Once inside the cell, the EB reorganizes within hours into the larger RB which divides repeatedly by binary fission and produces new infectious EBs.
- The EBs are then released by lysis of the host cell.

N.B.: The site of replication appears as an intra-cytoplasmic inclusion body, which can be stained and visualized microscopically.

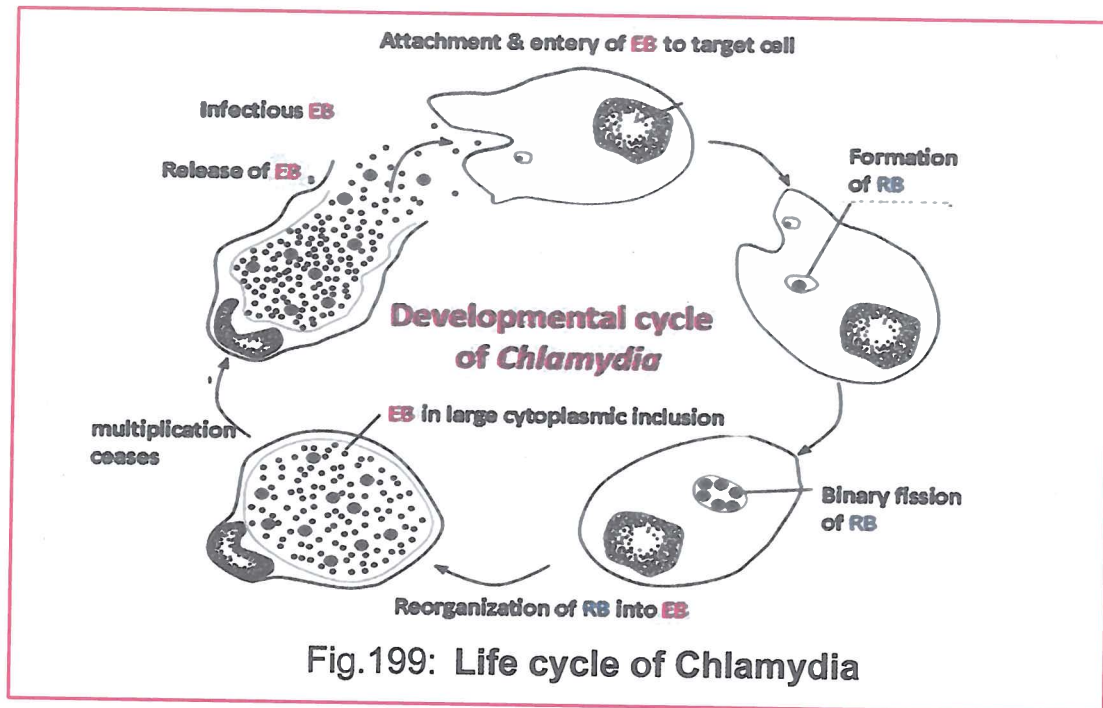


Fig.199: Life cycle of Chlamydia

**Chlamydiae that infect humans are divided into three species** (Table 15):

1. *Chlamydia trachomatis*: has several serotypes.
2. *Chlamydophila pneumoniae*.
3. *Chlamydophila psittaci*.

## ***Chlamydia trachomatis***

### **Diseases**

#### **I. Genital tract infections:**

These are sexually transmitted diseases.

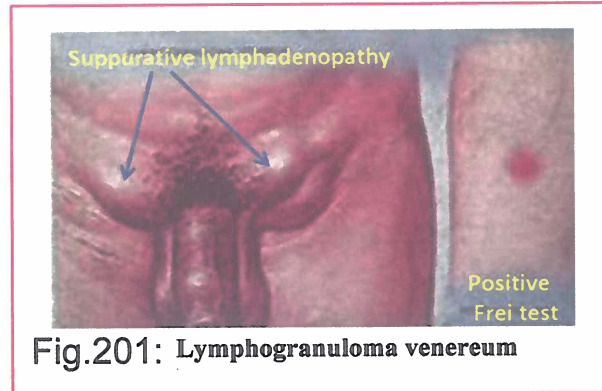
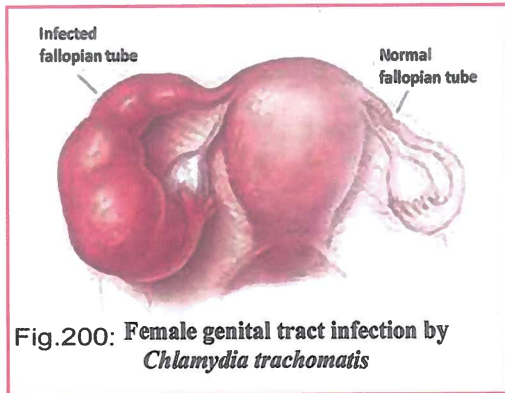
##### **1- Nongonococcal urethritis:**

- It is caused by serotypes D-K.
- Males present with urethritis, which may progress to epididymitis, prostatitis or proctitis.
- Females may present with urethritis and/or cervicitis, which may progress to salpingitis and pelvic inflammatory disease (PID). The condition may be complicated by infertility or ectopic pregnancy. (Fig.200)

N.B.: More than 50% of infected females are asymptomatic.

## 2- Lymphogranuloma venereum (LGV):

- It is caused by serotypes L1, L2 or L3.
- It manifests as an initial genital papule accompanied by suppurative inguinal lymphadenopathy. (Fig.201)



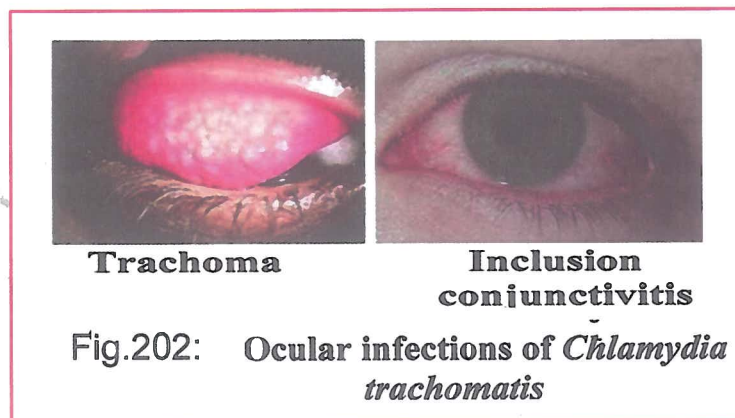
## II. Ocular infections: (Fig.202)

### 1- Trachoma:

- It is caused by serotypes A, B or C.
- Transmission commonly occurs by fingers, fomites and flies mostly during hot and dry climates.
- Trachoma manifests as chronic keratoconjunctivitis with scarring which may lead to blindness.
- Trachoma is an ancient disease which was well described in Egyptian writings around 3800 B.C.

### 2- Inclusion conjunctivitis:

- It is caused by serotypes D-K.
- It is the most common cause of neonatal conjunctivitis.
- Transmission occurs:
  - in neonates: during passage through an infected birth canal
  - in adults: as a result of transfer of organisms from the genitals to the eye (as an auto-infection) or through a vehicle (e.g., swimming pools).





### III. Neonatal pneumonia:

- It is caused by serotypes D-K.
- Infection occurs during passage through an infected birth canal.

### Laboratory diagnosis

**A. Specimens** are taken from urethra, cervix, conjunctiva, ..... etc.

**B. Direct detection**

- 1- Microscopic examination of Giemsa-stained smears for detection of the intra-cytoplasmic basophilic inclusion bodies. This method is insensitive, non-specific and requires good experience.
- 2- Antigen detection by immunofluorescence or by ELISA. This method is more specific, reliable and rapid.
- 3- Nucleic acid detection by DNA probes or PCR.

**C. Cultivation and identification:** Specimens are inoculated onto tissue culture. Intra-cytoplasmic inclusion bodies can be detected by Giemsa stain or immunofluorescence.

**D. Serological tests** are not helpful except in LGV infection.

**E. Frei skin test:** It is similar to tuberculin test. It is rarely used for diagnosis of LGV infection.

### Treatment and prevention

- The antibiotic of choice is erythromycin or tetracycline in adults.
- Erythromycin is recommended for neonates and pregnant women because of the effect of tetracycline on teeth and bones.
- Chlamydiae are not sensitive to  $\beta$ -lactam antibiotics due to lack of typical peptidoglycan layer.
- Detection and treatment of asymptomatic individuals are important preventive measures.

N.B.: It is recommended that patients receiving treatment for gonorrhoea also be treated with tetracycline for possible concurrent chlamydial infection.

## Chlamydophila

### • *Chlamydophila pneumoniae*

- This organism is transmitted from person to person by aerosols.
- It causes atypical pneumonia.
- It has been associated with atherosclerosis and asthma.

### • *Chlamydophila psittaci*

- *C. psittaci* is transmitted via inhalation of the organism in dried bird faeces or respiratory secretions; therefore, people handling birds regularly are particularly at risk.
- It causes psittacosis (zoonotic disease) which is an atypical pneumonia.

**Table (16): *Chlamydia* species of medical importance**

Species	Disease	Serotype	Transmission
<i>Chlamydia trachomatis</i>	<b>I. Genital infections:</b> Non gonococcal urethritis Lymphogranuloma venereum	D-K L1,L2,L3	Sexual Sexual
	<b>II. Ocular infections:</b> Trachoma Inclusion conjunctivitis: - in neonates - in adults	A,B,C D-K	Fingers, flies, fomite  - During birth - Swimming pool
	<b>III. Neonatal pneumonia</b>	D-K	- During birth
<i>Chlamydophila pneumoniae</i>	Atypical pneumonia Atherosclerosis Asthma	-	Inhalation of respiratory droplets from humans
<i>Chlamydophila psittaci</i>	Psittacosis = atypical pneumonia	-	Inhalation of particles contaminated from dried bird faeces or respiratory secretions

**MCQs:**

- 1- The following statements concerning *Chlamydia* are correct **EXCEPT**:
  - a- They are obligate intracellular parasites because they cannot synthesize sufficient energy.
  - b- They possess both DNA and RNA and are bounded by a cell wall.
  - c- *C. trachomatis* has multiple serotypes, but *C. psittaci* has only one serotype.
  - d- Some chlamydiae are transmitted by arthropods.
  - e- They multiply by binary fission.
  
- 2- What is the metabolically inert, infective form of chlamydia that can survive extracellularly?
  - a- Reticulate body
  - b- Virion body
  - c- Elementary body
  - d- Inclusion body
  - e- Negri body
  
- 3- The following diseases are caused by *Chlamydia trachomatis* **EXCEPT**:
  - a- Non-gonococcal urethritis
  - b- Psittacosis
  - c- Trachoma
  - d- Lymphogranuloma venereum
  - e- Inclusion conjunctivitis



RICKETTSIA

ILOs:

By the end of this chapter the student should be able to:

- Describe characteristic features of *Rickettsia*
- List the species of *Rickettsia* that are responsible for human infections
- Identify the mode of transmission, vectors, reservoirs and diseases of the various rickettsial species
- Describe the pathogenesis and clinical manifestations of various rickettsial diseases

Characteristic features

1. *Rickettsia* species are small obligate intracellular bacilli. They are unable to make sufficient ATP for independent life.
2. They have a Gram-negative cell wall structure. Rickettsiae are best stained by Giemsa stain.
3. They cannot grow on artificial laboratory media, but can be isolated by inoculation of laboratory animals or tissue cultures.
4. Organisms are maintained in nature by arthropods transmission.

Rickettsia species:

Common *Rickettsia* species are illustrated in (Table 17).

Table (17): Common *Rickettsia* species

Group	Organism	Disease	Arthropod vector	Mammalian reservoir	Geographic distribution	Clinical severity
Typhus group:	<i>R. prowazekii</i>	Epidemic typhus	Louse	Human	South America, Africa	++
	<i>R. typhi</i>	Endemic typhus	Flea	Rodents	Worldwide	-
	<i>Orientia tsutsugamushi</i>	Scrub typhus	Mite	Rodents	Far East	++
Spotted fever group:	<i>R. rickettsii</i>	Rocky Mountain spotted fever	Tick	Dogs, rodents	Rocky Mountain states, Eastern USA	+
	<i>R. akari</i>	Rickettsial pox	Mite	Mice	Asia, Far East USA	-
	<i>R. conorii</i>	Mediterranean Spotted fever	Tick	Dogs	Mediterranean	+

### Mode of transmission

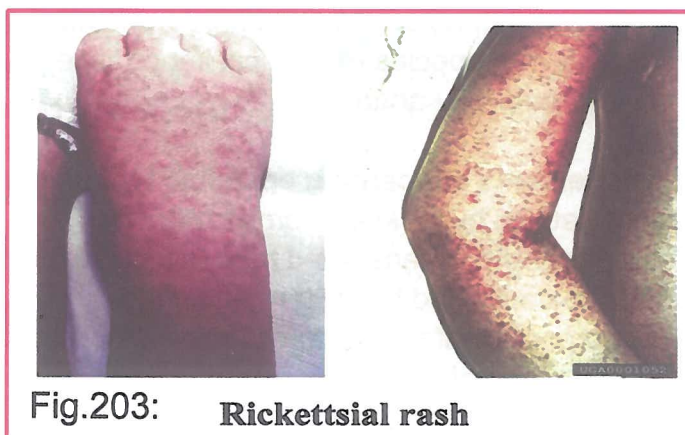
- Transmission of infection by infected **louse** or **flea** occurs by rubbing faeces of the vector into broken skin.
- Transmission of infection by infected **tick** or **mite** occurs via vector bite.
- In case of epidemic typhus, the infected lice are eventually killed by the infecting bacteria. Accordingly, this disease is not maintained in the lice population, i.e., there is no louse-to-louse transmission and human infection is an obligatory stage in the cycle.
- Ticks and mites can transmit the organism transovarially to their progeny and, thereby, the organism can be maintained in the tick or mite population without mammalian host for many years.

N.B.: All rickettsial diseases are considered to be zoonosis except epidemic typhus which occurs only in humans.

### Pathogenesis and clinical manifestations

- Rickettsiae infect the endothelial lining of the blood vessels, leading to vasculitis.
- Damage to the vessels of the skin results in the characteristic rash and in oedema and haemorrhage.
- Rickettsial disease is usually accompanied by fever, headache and skin rash.

(Fig.203)



- In severe cases, rickettsial encephalitis with coma, convulsions and pulmonary oedema are grave conditions that are often fatal.
- In some cases of epidemic typhus, rickettsiae are not eliminated from the body on clinical recovery and remain in the lymph nodes. As much as 50 years later, the infection can be reactivated to cause **Brill-Zinsser disease** (i.e., endogenous infection), and the patient once again acts as a source of infection for any louse that may be present.

### Laboratory diagnosis

Indirect immunofluorescent test is used to demonstrate anti- rickettsial antibodies.

### Treatment

Tetracycline is the drug of choice and chloramphenicol is an alternative.

### Prevention

Prevention is based on reducing exposure to the arthropod vector (e.g., delousing in case of epidemic typhus) and personal hygiene.

### MCQs:

- 1- Arthropods are vital in transmission of:
  - a- Mycoplasma
  - b- Chlamydia
  - c- Treponema
  - d- Rickettsia
  - e- Coxiella
- 2- Rickettsia are characterized by one of the following:
  - a- They are small extracellular bacilli.
  - b- They can be grown on artificial media.
  - c- They are maintained in nature by arthropod transmission.
  - d- They include only one species of medical importance.
  - e- They cannot be treated by antibiotics.
- 3- The following statements concerning epidemic typhus are correct **EXCEPT**:
  - a- The disease is caused by *Rickettsia prowazekii*.
  - b- The causative organism is transmitted from rodents to humans by ticks.
  - c- The disease is characterized by fever and skin rash.
  - d- The causative organism infects the vascular endothelial cells.
  - e- Tetracycline is the drug of choice.



## COXIELLA

### ILOs:

By the end of this chapter the student should be able to:

- List characteristic features differentiating *Coxiella* from *Rickettsia*
- List the species of *Coxiella* that is responsible for Q fever
- Describe the mode of transmission, clinical presentation, diagnosis & prevention of Q fever

*Coxiella* was formerly classified as *Rickettsia*. However, it differs from *Rickettsia* in the following:

- It is not transmitted to humans by arthropods.
- It is extremely resistant to heat, drying and sunlight, hence can persist outside the host for a long period.
- It mainly affects the lungs rather than vascular endothelium, so that infection is not accompanied by skin rash.

*Coxiella burnetii* is the only species of the genus *Coxiella*. It causes Q fever where Q stands for "Query" as the cause of fever was unknown for several years.

### Q Fever

Q fever is a zoonotic disease. The important reservoirs for human infection are cattle, sheep and goats; therefore, people handling infected animals (e.g., farmers, abattoir workers and veterinarians) are at high risk (occupational hazard).

### Mode of transmission

1. **Inhalation** of infected material (especially from faeces, placenta or urine of infected animals).
2. **Ingestion** of milk from infected animals.

### Clinical presentation

- In the majority of cases, infection remains asymptomatic.
- Clinical illness may be in the form of:
  - Fever and influenza-like symptoms.
  - Atypical pneumonia that may be complicated by hepatitis (the combination of pneumonia and hepatitis should suggest Q fever).
- Chronic Q fever is rare and usually manifests as endocarditis.

### Laboratory diagnosis

Diagnosis of Q fever depends upon serology because culture and molecular techniques have low sensitivity and are available only in reference laboratories.

## Treatment

Doxycycline is the drug of choice for treatment.

## Prevention

- Vaccination: Q fever vaccine used in humans consists of killed *C. burnetii* whole cells. It is usually given to people at risk.
- Pasteurization of milk.

**Table (18):** Comparison between *Mycoplasma*, *Chlamydia*, *Rickettsia*, *Coxiella* and viruses:

	<i>Mycoplasma</i>	<i>Chlamydia</i>	<i>Rickettsia</i>	<i>Coxiella</i>	Virus
Presence of RNA and DNA	Yes	Yes	Yes	Yes	No
Protein synthesis with own enzymes	Yes	Yes	Yes	Yes	No
Division by binary fission	Yes	Yes	Yes	Yes	No
Inhibition by antibiotics that stop protein synthesis	Yes	Yes	Yes	Yes	No
Growth on cell free media	Yes	No	No	No	No
Sterols requirement	Yes	No	No	No	No
Arthropod-borne transmission	No	No	Yes	No	ARBO Viruses

## MCQs:

- 1- *C. burnetii* is mainly transmitted by:
  - a- Ingestion of milk
  - b- Contact
  - c- Mosquito bites
  - d- Mite bite
  - e- Tick bite
- 2- *Coxiella burnetii*:
  - a- Is a type of extracellular bacteria
  - b- Has no reservoir other than humans
  - c- Causes a clinical condition called Q fever
  - d- Causes pneumonia only
  - e- Is easily stained by Gram stain

## ACTINOMYCETES

### ILOs:

**By the end of this chapter the student should be able to:**

- Describe the characteristic features of Actinomycetes
- Enumerate members of medical importance
- List morphology, oxygen requirement, habitat and disease caused by *Actinomyces israelii*
- Describe pathogenesis, clinical forms, diagnosis and treatment of actinomycosis
- List morphology, oxygen requirement, habitat and disease caused by *Nocardia asteroides*
- Describe pathogenesis, clinical forms, diagnosis and treatment of nocardiosis

### Characteristic Features

- Actinomycetes are true bacteria but they form long branching filaments that resemble hyphae of fungi. (Fig.204)
- They are Gram positive, but some are also weakly acid-fast.

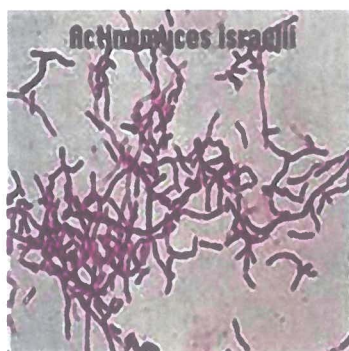


Fig.204: Actinomycetes (Gram stain)

### Members of medical importance

- Some members, particularly *Streptomyces* are important as producers of antimicrobial agents.
- Some members, such as *Nocardia brasiliensis*, cause mycetoma (actinomycetoma or actinomycotic mycetoma) see chapter 25.
- There are 2 important pathogenic species, *Actinomyces israelii* and *Nocardia asteroides*.



## *Actinomyces israelii*

*Actinomyces israelii* is an **anaerobe** that forms part of the normal flora of the oral cavity, intestinal tract and vagina. It causes **actinomycosis**.

### Pathogenesis

- Infection is **endogenous** following local trauma where the organism invades the tissues.
- A hard painless swelling develops, which drains pus through sinus tracts.
- The pus contains hard yellow granules (sulphur granules) which are composed of a mass of bacterial filaments.

### Clinical forms

- Cervicofacial actinomycosis (commonest type) where the face, neck and mandible are affected. It is usually related to poor dental hygiene and may be initiated by tooth extraction or some other trauma to the mouth or jaw. (Fig.205)
- Intestinal actinomycosis
- Pulmonary actinomycosis
- Actinomycosis of the skin



Fig.205: Cervicofacial actinomycosis

### Laboratory diagnosis

**Specimen:** Pus containing sulphur granules. The granules are crushed and subjected to:

1. **Microscopic examination**, which reveals Gram-positive branching filaments. (Fig.206)

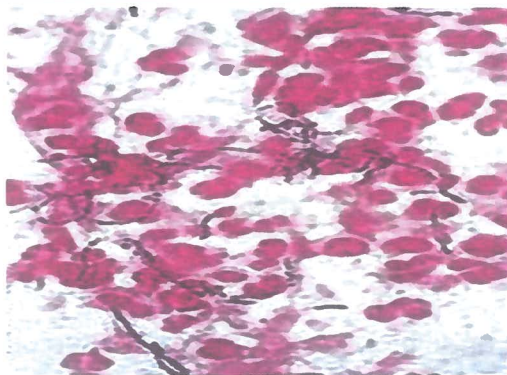
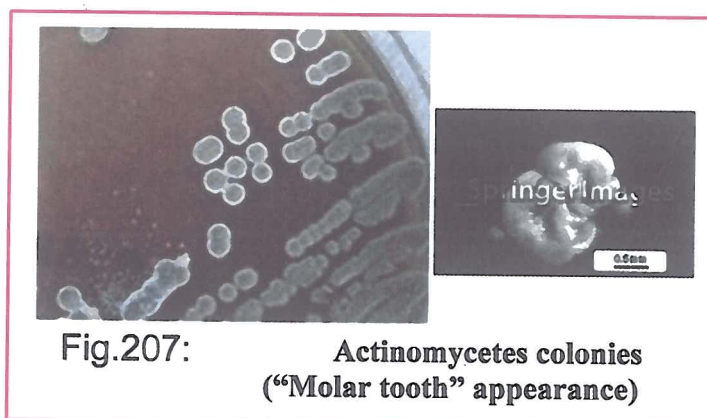


Fig.206: Actinomycetes (Gram stain)

2. **Culture** on blood agar under **anaerobic** conditions for about 2 weeks, which yields colonies with "molar tooth" appearance. The colonies are identified by morphology and immunofluorescence staining. (Fig.207)



### Treatment

1. Surgical drainage of pus.
2. Prolonged administration of penicillin, which is the drug of choice.

### *Nocardia asteroides*

Nocardiae are **aerobes** that are found in the environment particularly in the soil. *Nocardia asteroides* causes nocardiosis.

### Pathogenesis

- Infection is acquired from the soil by the airborne route or contamination of wounds.
- Nocardiae are opportunistic pathogens, infecting immunocompromised patients.

### Clinical forms

- **Pulmonary nocardiosis** which can be easily confused with tuberculosis or pulmonary malignancy. (Fig.208)
- **Brain abscess** which occurs through haematogenous spread from the lung. A combination of pneumonia and brain abscesses suggests *Nocardia* infection. (Fig.209)
- **Cutaneous nocardiosis** that manifests as pustules with tender regional lymphadenitis. (Fig.210)

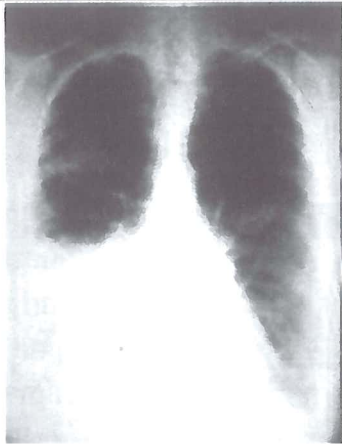


Fig.208: Pulmonary nocardiosis

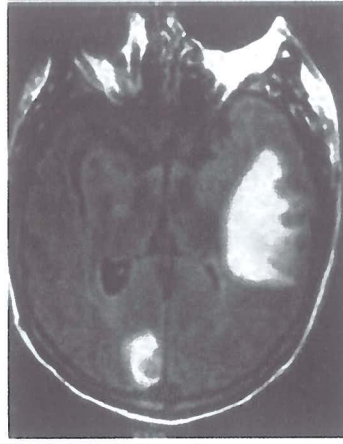


Fig.209: Brain abscess



Fig.210: Cutaneous nocardiosis

### Laboratory diagnosis

1. **Specimen:** According to the site of infection (i.e. sputum or pus).
2. **Microscopic examination** reveals Gram-positive branching filaments that are weakly acid fast. (Fig.211)

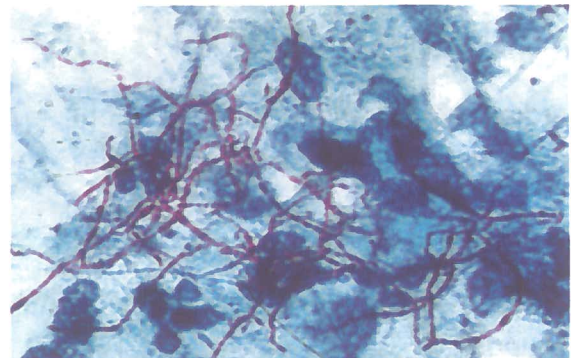


Fig.211: Nocardia (partially acid fast)

3. **Culture** on blood agar under aerobic conditions reveals bacterial growth after few days.

### Treatment

1. Trimethoprim-sulfamethoxazole for prolonged period (may be up to 1 year). The *Nocardia* are not susceptible to penicillin.
2. Surgical drainage may be required.



**Table (19):** Comparison between *A. israelii* and *N. asteroides*

Species	Habitat	Morphology	Oxygen requirement	Disease	Treatment
<i>Actinomyces israelii</i>	Oral cavity	Gram-positive branching filaments	Anaerobic	<ul style="list-style-type: none"> <li>• Actinomycosis: <ul style="list-style-type: none"> <li>- Cervicofacial</li> <li>- Intestinal</li> <li>- Pulmonary</li> <li>- Cutaneous</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Surgical drainage</li> <li>• Penicillin G</li> </ul>
<i>Nocardia asteroides</i>	Environment	Gram-positive branching filaments, weakly acid-fast	Aerobic	<ul style="list-style-type: none"> <li>• Nocardiosis: <ul style="list-style-type: none"> <li>- Pulmonary</li> <li>- Brain abscess</li> <li>- Cutaneous</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Sulfonamides</li> <li>• Surgical drainage</li> </ul>

**MCQs:**

- 1- The medical importance of *Streptomyces* is that:
  - a- It is used to test the autoclave efficiency.
  - b- It produces a large number of antibiotics.
  - c- It is an indicator of faecal pollution of water.
  - d- It has a probiotic effect.
  - e- It is a major part of the normal flora of the skin.
  
- 2- Pus containing sulphur granules is probably caused by:
  - a- *C. burnetti*
  - b- *R. prowazekii*
  - c- *Actinomyces israelii*
  - d- *Candida albicans*
  - e- *Chlamydia*
  
- 3- *Nocardia asteroides* causes the following infections **EXCEPT**:
  - a- Pulmonary infection
  - b- Brain abscess
  - c- Cutaneous infection
  - d- Regional lymphadenitis
  - e- Genital infection

# Answers

Chapter 1:	1 c	2 c								
Chapter 2:	1 c	2 c	3 b	4 c						
Chapter 3:	1 e	2 d	3 a	4 d	5 a					
Chapter 4:	1 e									
Chapter 5:	1 d	2 c	3 b	4 d						
Chapter 6:	1 d	2 b	3 c	4 b	5d					
Chapter 7:	1 d	2 d	3 d	4 d	5 c	6 d				
Chapter 8:	1 e	2 b	3 e	4 b	5 d	6 b	7 d	8 c		
Chapter 9:	1 c	2 d	3 d	4 e						
Chapter 10:	1 c	2 b	3: a F	b T	c F	d T	e F	f T	g T	h
Chapter 11:	1 c	2 e	3 b							
Chapter 12:	1 d	2 b								
Chapter 13:	1 b	2 d	3 e	4 a	5 d					
Chapter 14:	1 c	2 a	3 d							
Chapter 15:	1 c	2 c	3 c	4 c						
Chapter 16:	1 c	2 d								
Chapter 17:	1 d	2 c	3 d							
Chapter 18:	1 e	2 e	3 e	4 e	5 c	6 e	7e			
Chapter 19:	1 b	2 d	3 c	4 b	5 d	6 e				
Chapter 20:	1 c	2 c	3 b							
Chapter 21:	1 d	2 c	3 b							
Chapter 22:	1 d	2 c	3 b							
Chapter 23:	1 a	2 c								
Chapter 24:	1 b	2 c	3 e							

# ESSENTIAL MEDICAL MICROBIOLOGY and IMMUNOLOGY



Volume IV





**ESSENTIAL  
MEDICAL MICROBIOLOGY  
AND IMMUNOLOGY**

**VOLUME IV**

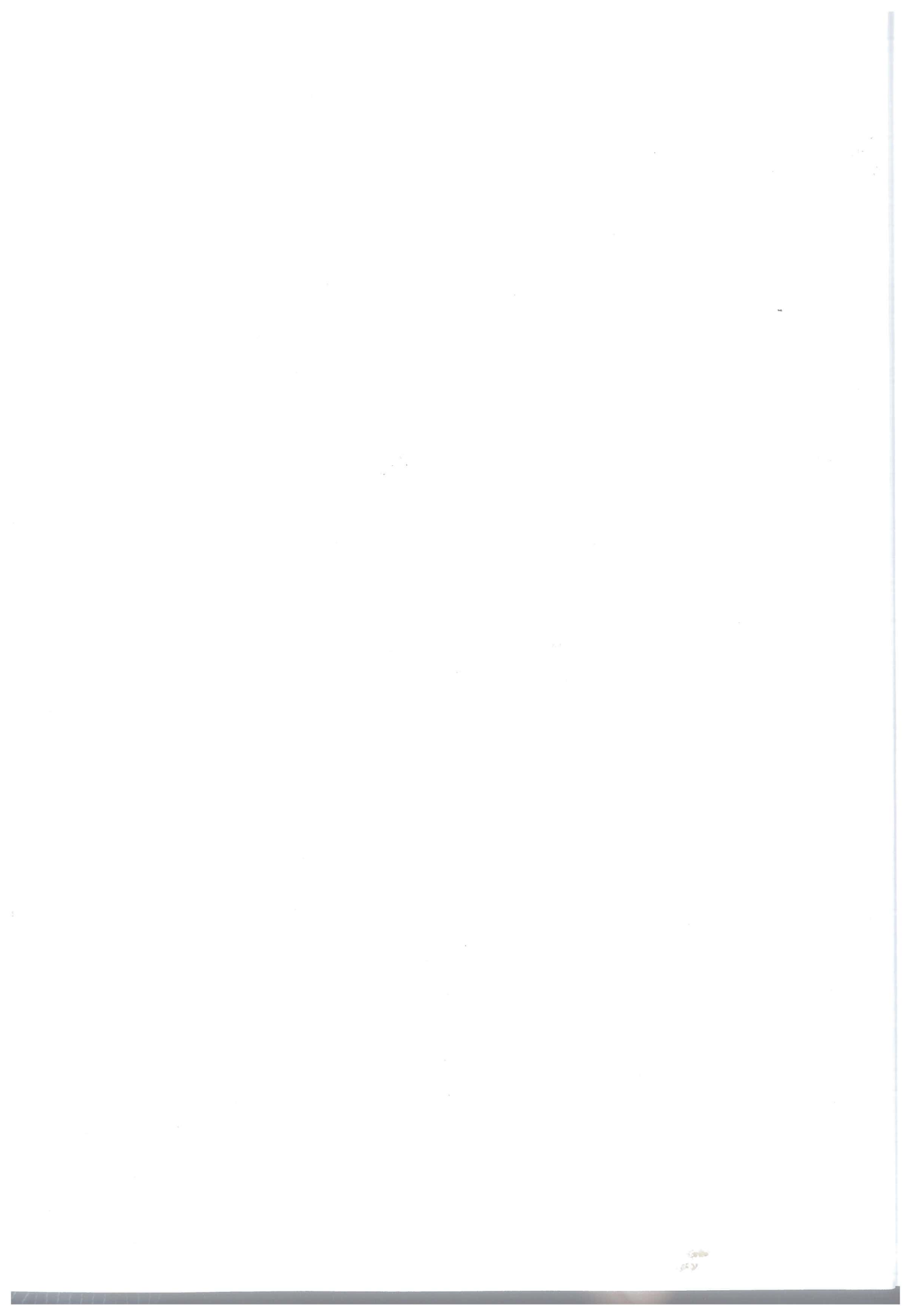
**Systematic Virology  
Systematic Mycology  
Applied Microbiology**

**Eighth Edition**

**By**

***Staff Members of  
Medical Microbiology and Immunology Department***

**Faculty of Medicine-Cairo University  
2018-2019**





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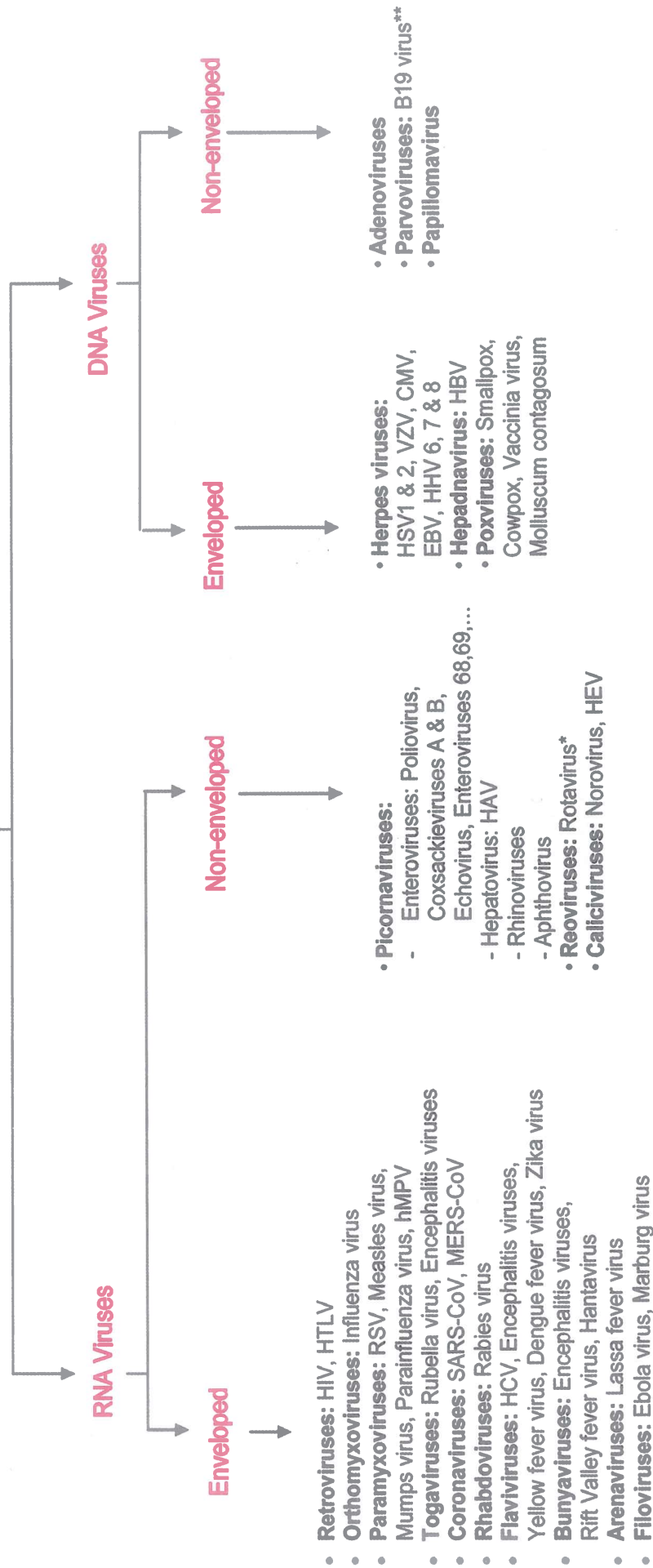
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# **SYSTEMATIC VIROLOGY**



## Classification of Viruses



### N.B.:

- Hepatitis viruses (A-E) belong to different families.
- Arboviruses include members in *Flavi-*, *Toga-* and *Bunyaviridae* families.
- Roboviruses include members in *Bunyaviridae* and *Arenaviridae* families.
- Tumour viruses are present among different families

\* Rotavirus is the only double-stranded RNA virus.

\*\* Parvoviruses are the only single-stranded DNA viruses.

## HEPATITIS VIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- List different viruses that cause infection of the liver and classify their taxonomy
- Identify the structure of HAV, HBV, HCV, HDV, HEV
- List the mode(s) of transmission of HAV, HBV, HCV, HDV, HEV
- Explain the pathogenesis of HAV, HBV, HCV, HDV, HEV
- State the clinical presentation(s) of HAV, HBV, HCV, HDV, HEV and mention the outcome of infection caused by them
- Identify the laboratory diagnosis of HAV, HBV, HCV, HDV, HEV
- Summarize hepatitis B virus markers and evaluate their significance
- List and evaluate the available methods used for the prevention of HAV, HBV, HCV, HDV, HEV infections
- Summarize the treatment of HAV, HBV, HCV, HDV, HEV infections
- Identify HAV vaccine and HBV vaccine and state their nature, route and schedule of administration

Many viruses cause hepatitis. Of these, five medically important viruses are commonly described as “hepatitis viruses” because their main site of infection is the liver. These five are hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV, delta virus) and hepatitis E virus (HEV). These viruses belong to different families. Important properties of these hepatitis viruses are illustrated in table (1).

**Table (1):** Hepatitis viruses

Virus	HAV	HEV	HBV	HDV	HCV
Family	Picornavirus	Calicivirus	Hepadnavirus	Deltavirus	Flavivirus
Genome	ssRNA	ssRNA	dsDNA	ssRNA	ssRNA
Envelope	No	No	Yes	Yes	Yes
HBsAg in envelope	No	No	Yes	Yes	No

N.B.: Other viruses, such as Epstein-Barr virus, cytomegalovirus and yellow fever virus infect the liver but also infect other sites in the body and therefore are not exclusively hepatitis viruses.

## Hepatitis A Virus (HAV)

- HAV is a non-enveloped ssRNA virus (Table 1).
- It causes hepatitis A which has been called infectious hepatitis in the past.
- The disease occurs in sporadic or epidemic forms. It affects children and young adults.
- There is only one serotype and development of antibodies to HAV confers lifelong immunity.

### Pathogenesis (Fig. 1)

- Humans are the reservoir for HAV.
- The virus is transmitted mainly by the faeco-oral route usually through ingestion of contaminated food or water.
- The virus replicates in the GIT and spreads via the blood to the liver, where it replicates in the hepatocytes and is then shed in bile resulting in excretion of large amounts of the virus in faeces.

N.B.: HAV is rarely transmitted via the blood, due to short duration and low level of viraemia.

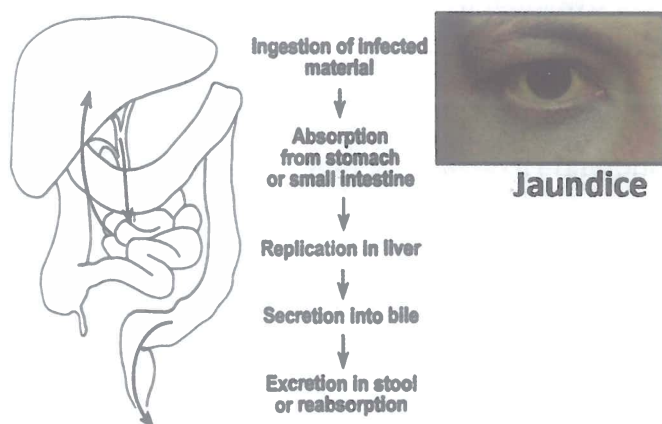


Fig. (1): Pathogenesis of HAV infection

### Clinical features

- Most HAV infections are asymptomatic or subclinical.
- Fever, anorexia, nausea, vomiting and dark urine are typical manifestations. These manifestations are the same regardless of which hepatitis virus is the cause.
- The incubation period is 2-6 weeks.
- HAV infection is usually mild self-limited and does not progress to chronic hepatitis
- Infection is followed by long-lasting immunity (mediated by IgG).

### Laboratory diagnosis (Fig. 2)

1. Marked elevation of liver transaminases and bilirubin.
2. Detection of anti-HAV IgM is diagnostic of acute illness.



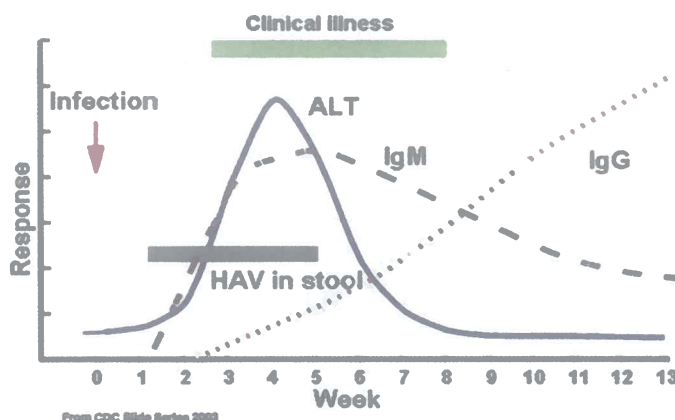


Fig. (2): Serologic course of HAV infection

## Prevention and control

### I. General measures:

- Proper hand-washing, chlorination or boiling of drinking water.
- Careful disposal of sewage and avoiding contamination of food and water.

### II. Specific prophylaxis:

- **Active immunization:**
  - An inactivated vaccine (Havrix) can be given intramuscularly as two doses at 0 and 6 months. It is recommended to high-risk individuals including children above 2 years of age who live in endemic countries.
  - A combination vaccine (Twinrix) that immunizes against HAV and HBV is available.
- **Passive immunization:**
  - HAV immunoglobulin may be given for post-exposure prophylaxis to prevent disease in immune-deficient individuals.

## Hepatitis E Virus (HEV)

- HEV is a non-enveloped ssRNA virus (Table 1).
- HEV is epidemiologically and clinically similar to HAV with the exception of a high mortality rate in pregnant females due to fulminant hepatitis.
- Diagnosis is made by detection of anti-hepatitis E virus IgM.

## Hepatitis B Virus (HBV)

- HBV is an enveloped dsDNA virus (Table 1).
- The virion, historically known as the **Dane particle**, is spherical with 42 nm diameter (Fig. 3)
- The envelope contains a protein called the surface antigen (**HBsAg**). HBV expresses excess HBsAg and releases it into the bloodstream as independent particles. There are two forms of such particles, 22 nm spherical and 200 nm tubular particles (Fig. 4). Because there is no viral DNA in these particles, they are not infectious.

- The nucleocapsid contains 2 additional antigens:
  - The core antigen (**HBcAg**) which is confined to the liver cells.
  - The e antigen (**HBeAg**) which is secreted from infected cells into the blood.

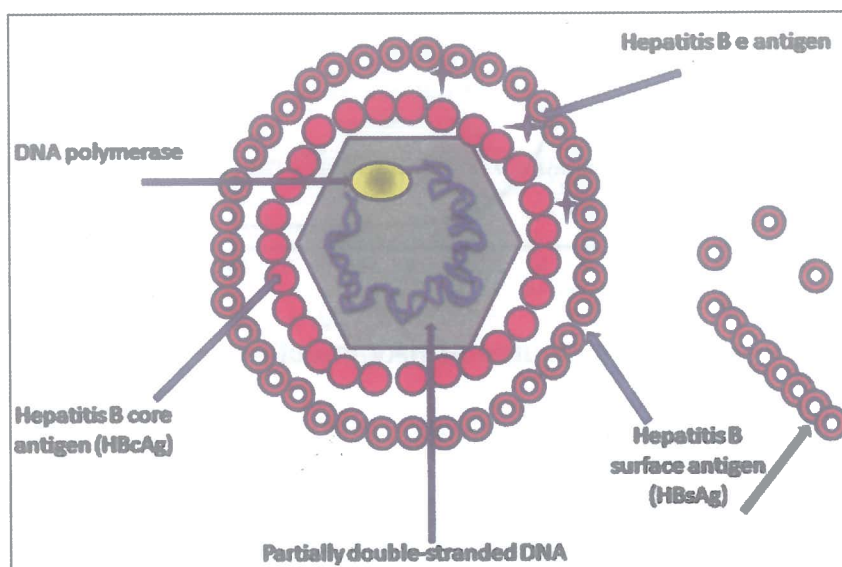


Fig. (3): Structure of HBV

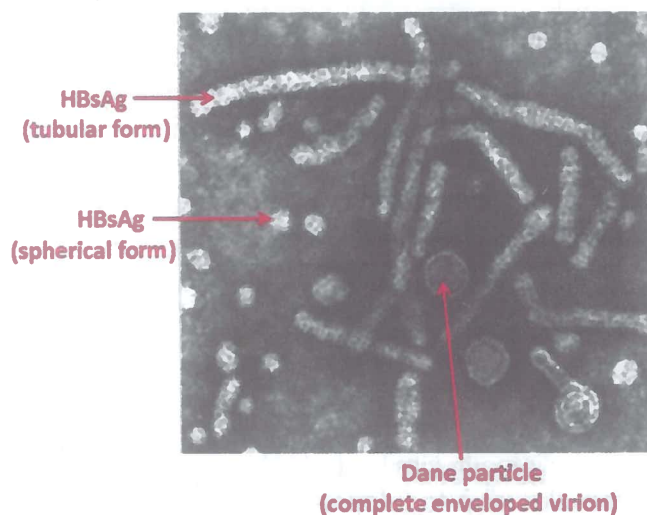


Fig. (4): HBV (E/M)

### Modes of transmission

- HBV is present in all body fluids of infected individuals (blood, semen, saliva...etc)
- The main routes of infection are:
  - blood and percutaneous transmission
  - sexual transmission
  - perinatal transmission

N.B.: Trans-placental transmission accounts for a minority of cases (especially with high maternal viral load).
- Because the viral load may reach very high levels (up to  $10^{10}$  copies/ml serum), the possibility of transmission may be very high compared to the blood-borne viruses HIV and HCV.

## Pathogenesis

- After entering the body, the virus reaches the blood then the liver resulting in inflammation and necrosis.
- HBV itself does not cause a cytopathic effect.
- The primary cause of hepatic cell destruction appears to be immune-mediated by cytotoxic T-cells which react specifically with viral antigens displayed on the surface of infected hepatocytes.
- Humoral immune response to HBsAg will result in the formation of HBsAb. This will complex with the excess HBsAg forming immune complexes resulting in:
  - extra-hepatic manifestations in 10-20% of patients due to deposition of the immune complexes mainly in the skin, joints and glomeruli,
  - undetectable HBsAb in serum as it is consumed in the immune complexes.

## Clinical manifestations

- Many HBV infections are asymptomatic.
- In symptomatic patients manifestations appear after an incubation period of 6 weeks to 6 months.
- Clinical symptoms and signs include nausea, vomiting and abdominal pain. The liver is enlarged and tender. Malaise and anorexia may precede jaundice.
- Extra-hepatic manifestations include skin rash, urticaria, polyarthralgia and glomerulonephritis.

## Clinical outcomes of acute HBV infection

- **Full recovery:**
  - It occurs in most patients within 4-6 months.
  - It is due to effective cell mediated and humoral immune responses.
- **Fulminant hepatitis:**
  - It occurs in 1-2% of patients.
  - It may be due to massive lysis of hepatocytes by vigorous immune response, a more highly virulent strain of HBV or co-infection with another hepatitis virus (e.g., HCV or HDV).
- **Chronic infection:** (Table 2)
  - It occurs in 5-10% of patients.
  - It is due to limited cell-mediated and humoral immune responses.
  - It may be in the form of:
    - 1- **Chronic hepatitis:** It may end in cirrhosis and hepatocellular carcinoma.
    - 2- **Chronic carriers:** These are asymptomatic patients with persistent HBsAg for more than 6 months. Their blood remains infectious often for life.

## HBV infection in neonates

The immune system of the newborn is less competent than that of the adult, therefore:

- Perinatally infected infants generally have no clinical signs or symptoms, with normal levels of liver enzymes.
- Chronic carriage is more likely to occur (approximately 90%), with a higher risk of hepatocellular carcinoma (HCC).



**Table (2):** Comparison between chronic hepatitis and chronic carriers

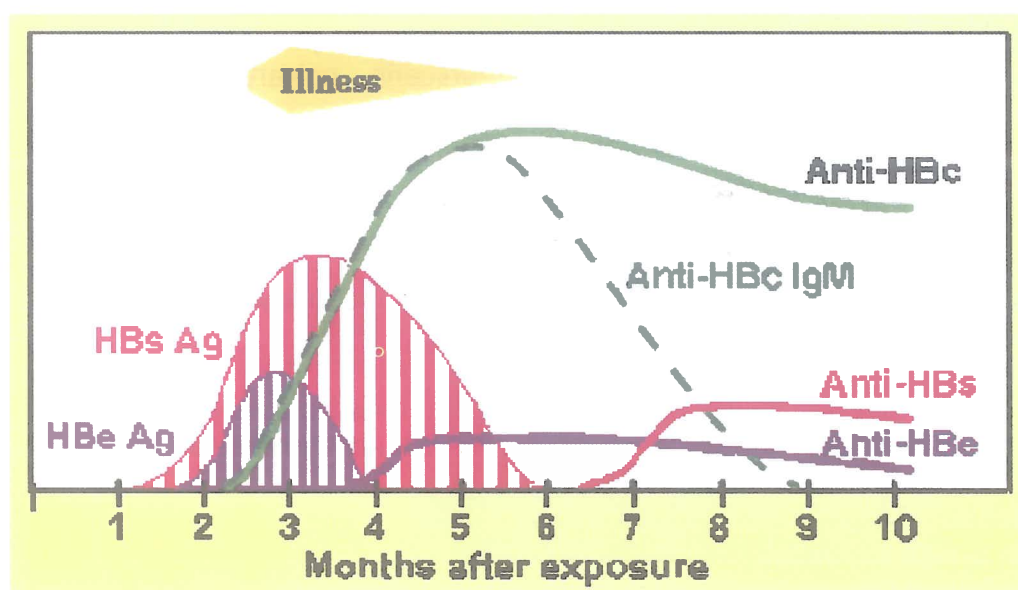
	Chronic hepatitis	Chronic carriers
Persistence of HBsAg	for > 6 months	for > 6 months
Liver enzymes (ALT/AST)	elevated (persistently or intermittently)	normal
Viral load	high	low or undetectable
HBeAg	may be present (especially with higher viral load)	usually absent
Liver biopsy	chronic hepatitis with moderate or severe inflammation & necrosis	absence of significant hepatitis
Outcome	may end in cirrhosis and HCC	may remain apparently healthy for life or progress to chronic hepatitis

### Laboratory diagnosis

#### A) Liver function tests:

Serum alanine aminotransferase (ALT) and bilirubin are markedly elevated.

#### B) Hepatitis B virus markers: These are HBV antigens and antibodies that can be detected in the patients sera (Fig. 5):

**Fig. (5):** Typical serologic course of HBV infection

#### 1. HBsAg:

- It can be detected during the incubation period and active disease.
- It usually declines within a period of 12 weeks.
- Its persistence for more than 6 months indicates chronicity.

#### 2. HBsAb:

- It becomes detectable after the disappearance of HBsAg.
- HBsAb is in fact being made during the acute disease but is undetectable because it is bound in immune complexes.
- Similarly, it is not detectable in the chronic state due to persistence of HBsAg.
- Its presence denotes immunity because HBsAb neutralizes the infectivity of HBV.

3. **HBcAg:**

It is present only in the liver cells, and can not be detected in blood.

4. **HBcAb:**

- **IgM** form of HBcAb is found during the acute stage and disappears approximately 6 months after infection; thus, it is an indicator of recent HBV infection.
- As IgM wanes, HBcAb of the **IgG** form is detected and persists for life whether the patient recovers from acute infection or develops chronic hepatitis; thus, it is an indicator of past infection.
- There is a period designated the **window phase** during which HBsAg has disappeared while anti-HBs is not yet detectable. During this phase, IgM HBcAb is always positive and diagnostic.

5. **HBeAg:**

- It can be detected in the serum in the late incubation period and during the acute illness.
- Its presence is associated with high infectivity of the patient.

6. **HBeAb:**

It becomes detectable during convalescence and its presence is an indicator of low infectivity.

C) **Viral DNA:**

It is an indicator of viral replication and is important in diagnosis of chronic infection.

**Table (3): Interpretation of hepatitis B markers**

Tests	Results	Typical interpretation
HBsAg HBcAb (IgM) HBsAb	Positive Positive Negative	<b>Acute</b> HBV infection
HBsAg HBcAb (IgM) HBsAb	Negative Positive Negative	<b>Window phase</b>
HBsAg HBcAb (IgG) HBcAb (IgM) HBsAb	Positive Positive Negative Negative	<b>Chronic</b> HBV infection
HBsAg HBcAb (IgG) HBsAb	Negative Positive Positive	<b>Immunity</b> following natural infection
HBsAg HBcAb HBsAb	Negative Negative Positive	<b>Immunity</b> following HBV vaccination

## Treatment

- Specific treatment for acute HB illness is usually not needed because in the majority of cases the immune system controls the infection and eliminates the virus within 6 months.
- The goal for treatment in patients with chronic hepatitis B is to reduce the risk of progressive liver disease and complications.
- The most commonly used drugs are interferon- $\alpha$  or a nucleoside analogue e.g., lamivudine. However, these drugs do not cure HB infection; in most patients when the drug is stopped HB virus replication resumes.

## Prevention and control

### 1. General measures:

- a- Screening of blood before transfusion.
- b- Applying the infection control practices.

### 2. Specific prophylaxis:

#### a- Hepatitis B vaccine (active immunization):

- This vaccine contains HBsAg produced in yeast by recombinant DNA technique.
- HB vaccination is now recommended as a routine infant immunization at 2, 4 and 6 months of age. It is also indicated in a series of 3 intramuscular doses at 0, 1 and 6 months for people who may be exposed to blood and blood products (e.g., healthcare workers, medical students, dialysis patients .... etc.)

#### b- Hepatitis B immunoglobulin (passive immunization):

- HB immunoglobulin is prepared from sera of patients who have recovered from hepatitis B (convalescent serum). It contains high titre of HBsAb.
- It is indicated **together with vaccination** for:
  - 1. Neonates born to HBsAg positive mothers.
  - 2. Unvaccinated individuals (or those with incomplete vaccination) accidentally exposed to HBV (e.g., needle-stick injury).

## Hepatitis D Virus (Delta Virus)

- HDV is an enveloped ssRNA virus (Table 1).
- It is also called delta virus because it has an internal core protein known as delta antigen.
- It is a **defective** virus, i.e. cannot replicate by itself because it does not have the genes for its envelope protein. It replicates only in the presence of HBV (as a helper virus) which provides HDV with the envelope protein (HBsAg).
- Its mode of transmission is similar to that of HBV. Infection is either a co-infection where the patient acquires both viruses at the same time or a superinfection, i.e., on top of a chronic HBV infection where it may lead to more severe acute disease with greater risk of complications.
- Diagnosis can be made by detection of:
  - HDV antibodies
  - delta antigen
  - viral RNA by PCR
- HDV can be controlled through control of hepatitis B infection.



## Hepatitis C Virus (HCV)

- HCV is an enveloped ssRNA virus (Table 1).
- It has 6 genotypes (1-6) and multiple subtypes.
- HCV infection is a growing major health problem worldwide. In Egypt, the prevalence of HCV infection ranges between 10-25% according to different locations, where genotype 4 causes the majority of cases.

### Mode of transmission

**Parenteral route:** exposure to contaminated blood; for example:

- In drug users, tattooing, ear piercing, sharing razors.....etc.
- Contaminated haemodialysis equipment.
- Transmission by needle-stick injury (e.g., in healthcare workers). However, the risk is lower than HBV.
- Transmission via transfusion of blood and blood products rarely occurs nowadays due to routine screening.

N.B.:

1. Unlike HBV, HCV transmission is **uncommon** vertically (from infected mother to infants) and sexually.
2. The route of transmission is **unknown** in up to 40% of infected individuals.

### Pathogenesis

- In the HCV infected individuals, viral replication occurs in the hepatocytes.
- Destruction of liver cells may result from the host immune response including cytotoxic T cells.

### Clinical manifestations (Fig. 6)

- The majority of HCV infections are subclinical, but about 20% of individuals present with acute hepatitis after an incubation period of about 4-6 months.
- Spontaneous resolution may occur following acute infection.
- A significant proportion of infected individuals progress to chronic hepatitis that may end in cirrhosis or hepatocellular carcinoma.
- Unlike HBV:
  - Clinical manifestations of acute HCV are milder.
  - Chronic carriage state occurs more often (~ 80%).

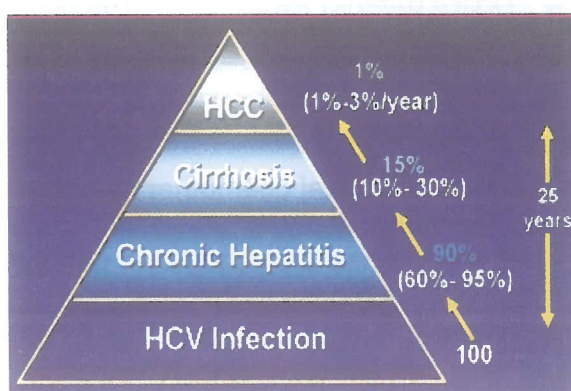


Fig. (6): Clinical course of HCV infection

### Laboratory diagnosis (Fig. 7, Table 4)

1. Detection of anti-HCV antibodies by ELISA, or the more specific RIBA test (recombinant immunoblotting assay). Seroconversion may take up to 6 months.
2. Detection of viral RNA in blood by PCR. This is useful in:
  - a) Diagnosis of early cases before seroconversion.
  - b) Serologically positive cases to detect the presence of viral RNA and, thus, the need for therapy.
  - c) Follow up the response to treatment (by measuring the viral load).
  - d) Genotyping of HCV: The genotype is the strongest predictor of response to interferon and ribavirin therapy and thus is routinely determined during the evaluation of patients.

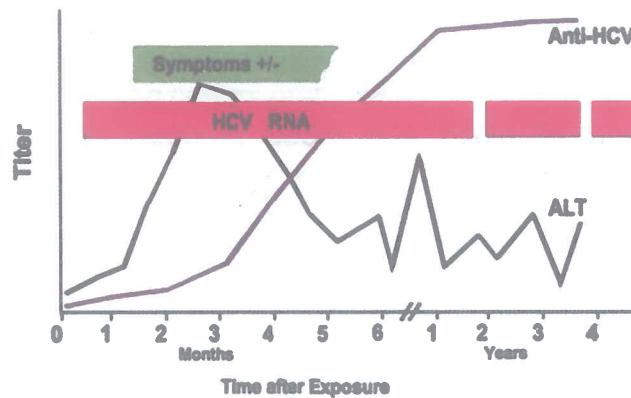


Fig. (7): Laboratory results during HCV infection

Table (4): Interpretation of tests used in the diagnosis of HCV infection

Anti-HCV EIA*	HCV RNA**	Interpretation
Negative	Negative	Not infected
Positive	Negative	Resolved HCV infection
Negative	Positive	Early acute HCV infection or chronic HCV infection in immune compromised person
Positive	Positive	Acute or chronic HCV infection

### Treatment

- A combination of alpha-interferon and antiviral chemotherapy (ribavirin) has been used. Response depends upon the virus genotype:
  - Genotypes 2 and 3 have better response to therapy than genotype 1.
  - Unfortunately genotype 4 has poor response to therapy.
- Sofosbuvir (Sovaldi) is a nucleotide analogue inhibitor. It is more effective with shorter treatment duration and less side effects. It is used with:
  - Ribavirin for treatment of genotypes 2 and 3.
  - Interferon for treatment of genotypes 1 and 4.

## Prevention and Control

- No specific vaccine or immunoglobulin is available for immunoprophylaxis.
- General measures directed at preventing exposure to the virus are the same as those for HBV.
- In case of exposure, follow-up is recommended by screening for HCV-RNA or anti-HCV for up to 6 months.

**Table (5): Hepatitis viruses**

Virus	HAV	HEV	HBV	HDV	HCV
Mode of transmission	Faeco-oral	Faeco-oral	- Parenteral - Sexual - Perinatal	Parenteral (Super or co-infection with HBV)	Parenteral
Disease presentation:					
- Acute stage:	Mild	Mild (severe in pregnancy)	Occasionally severe	Usually severe	Usually subclinical
- Chronic stage:	No chronicity	No chronicity	10% in adults, 90% in infants	As HBV	80% of cases
- Sequelae:	No sequelae	No sequelae	Cirrhosis, HCC, fulminant hepatitis	Cirrhosis, fulminant hepatitis	Cirrhosis, HCC
Mortality	<0.5%	~ 1% (up to 25% during pregnancy)	~ 1%	High	~ 1%
Laboratory diagnosis	HAV IgM	HEV Ab	HBsAg, HBcAb (IgM)	HDV Ab, HBsAg	HCV Ab, HCV RNA
Active immunization	Inactivated vaccine	—	Recombinant vaccine	—	—
Passive immunization	HAV-Ig	—	HBV-Ig	—	—



**MCQs:**

- 1- Regarding hepatitis A viral infection:**
  - a- It can be prevented by an inactivated vaccine.
  - b- Liver enzymes are usually decreased.
  - c- Diagnosis is routinely done by isolating the virus in cell culture.
  - d- It usually causes a chronic form of hepatitis that ends in liver cirrhosis.
  - e- It is commonly acquired through contact with blood from an infected person.
  
- 2- The hepatitis virus which is acquired faeco-orally and causes a high mortality rate in pregnant women is:**
  - a- Hepatitis A virus
  - b- Hepatitis B virus
  - c- Hepatitis C virus
  - d- Hepatitis D virus
  - e- Hepatitis E virus
  
- 3- The marker that is closely associated with HBV infectivity is:**
  - a- HBsAg
  - b- HBeAg
  - c- Anti-HBs
  - d- Anti-HBe
  - e- Anti-HBc
  
- 4- A patient with HBV infection tested positive for HBsAg, negative for anti-HBsAbs, positive for anti-HBcAbs of IgG type and negative for anti-HBcAbs of IgM type. The diagnosis of this patient is most probably:**
  - a- Acute HBV infection
  - b- Immune state following vaccination
  - c- Immune state following natural infection
  - d- Window phase
  - e- Chronic HBV infection
  
- 5- All of the following statements about HCV are correct EXCEPT:**
  - a- HCV-infected patients are predisposed to hepatocellular carcinoma.
  - b- HCV is an important cause of post-transfusion hepatitis.
  - c- An inactivated vaccine prevents the disease in exposed individuals.
  - d- Diagnosis is made by detecting anti-HCV antibodies by ELISA.
  - e- Alpha interferon + ribavirin (antiviral chemotherapy) are used in treatment.

## RETROVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- List the important members of *Retroviridae* family and their characteristics
- Identify and illustrate the structure of HIV
- Explain the pathogenesis of HIV
- State and explain the clinical findings of HIV infection
- Summarize the laboratory diagnosis of HIV infection
- List the different types of antiretroviral drugs, their mechanism of action and methods used for monitoring anti-HIV therapy

- Retroviruses are **enveloped positive sense ssRNA** viruses.
- They are so called because they contain the enzyme **reverse transcriptase** (Latin: Retro = backwards/reverse) which can synthesize complementary DNA from RNA. This enzyme is responsible for a unique feature of replication not found in other viruses.
- Retroviruses of medical importance include:
  1. **Lentiviruses:** These are cytocidal slow viruses. They include:
    - Human immunodeficiency virus-1 (HIV-1)
    - Human immunodeficiency virus-2 (HIV-2)
  2. **Deltaretroviruses:** These are oncoviruses. They include:
    - Human T-cell lymphotropic virus-1 (HTLV-1)
    - Human T-cell lymphotropic virus-2 (HTLV-2)

## Human Immunodeficiency Viruses

- Since the initial description of HIV-1 in 1983 and HIV-2 in 1986, these two viruses have been identified as the primary cause of the **acquired immunodeficiency syndrome (AIDS)**.
- HIV-1 is the major cause of AIDS worldwide, while AIDS caused by HIV-2 is limited mostly to west Africa; in addition it is much less severe and slower in progression.

### Structure

HIV is a medium-sized spherical virus. It has a cylindrical (bar-shaped) core that contains the viral genome and is surrounded by an envelope (Fig. 8).

#### The viral envelope:

It is composed of a lipid membrane and contains 2 virus-specific glycoproteins: gp120 and gp41.

- a) **gp120** protrudes from the surface and is responsible for viral binding to host cell receptors.
- b) **gp41** is embedded in the envelope and mediates the fusion of the viral envelope with the cell membrane at the time of infection.

#### The viral genome:

It consists of 2 identical copies of (+) sense ssRNA, each of which has a copy of the virus nine genes: 3 major (structural) genes (*env*, *pol* & *gag*) and 6 regulatory genes.

- a) **env gene** (envelope): encodes gp160, a precursor glycoprotein that is cleaved to form the 2 envelope glycoproteins: gp120 and gp41. Rapid mutation in this gene results in many gp120 antigenic variants.
- b) **pol gene** (polymerase): encodes the enzymes reverse transcriptase, integrase and protease which participate in viral replication.
- c) **gag gene** (group specific antigen): encodes the core proteins, the most important of which is p24.

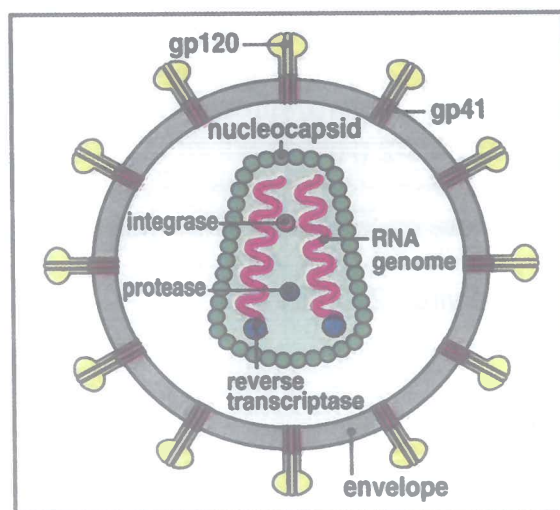


Fig. (8): Structure of HIV



## Pathogenesis

### • HIV transmission:

HIV transmission occurs when a person is exposed to body fluids contaminated with the virus, such as blood, semen, vaginal secretions and breast milk. The primary modes of HIV transmission are:

1) **Sexual transmission:** sexual contact (hetero- or homosexual) with an infected person.

2) **Parenteral transmission:**

- e.g. sharing needles or accidental pricking by a needle contaminated with infected blood. For this reason health care workers and drug addicts are among risk groups.
- Routine screening of blood for antibodies to HIV markedly reduced the risk of acquiring HIV from blood transfusions.

3) **Vertical transmission:** approximately 25% of HIV-infected pregnant women who are not treated during pregnancy, can transmit HIV to their infants during pregnancy, childbirth or through breast-feeding.

### • Cells infected by HIV:

- CD4 T helper cells.
- Macrophages and monocytes.
- Dendritic cells.
- Oligodendrocytes, astrocytes, neurons and glial cells.
- Follicular dendritic cells (in lymph nodes).

Except for the CD4 T lymphocytes, these cells are not necessarily destroyed by the virus; therefore they act as reservoir for further T cell infection.

- Following sexual transmission, the dendritic cells in the genital mucosa carry the virus from the site of infection to the lymph nodes where other cells (esp. T helper cells) become infected (Fig. 9).
- Infection of CD4 T helper cells leads to their depletion by 2 main mechanisms:
  - direct killing of infected cells by the virus as a result of viral replication,
  - killing of infected CD4 T cells by CD8 cytotoxic lymphocytes.
- The depletion of T helper cells leads to loss of cell-mediated immunity, increased susceptibility to infections and malignancies, and eventually death.

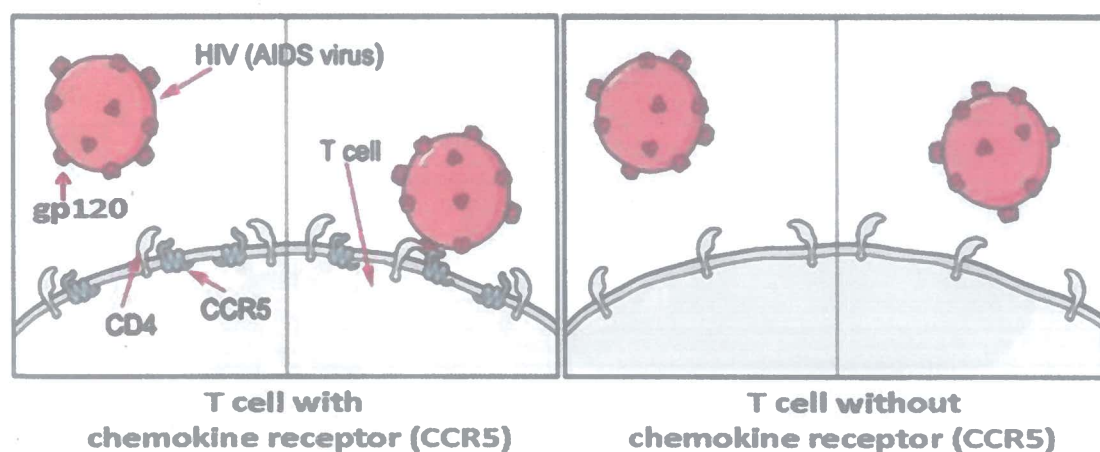


Fig. (9): HIV binding to CD4 T cell

**Replication of HIV in infected cells (Fig. 10):**

The replication of HIV follows the typical retroviral cycle:

**1) Binding (attachment):**

The viral gp120 binds to CD4 molecules followed by binding to a chemokine receptor on the cell surface. Genetic deficiency of this chemokine receptor results in resistance to HIV infection (Fig. 10).

**2) Penetration:**

The viral gp41 mediates fusion of the viral envelope with the cell membrane, and the virus enters the cell.

**3) Reverse Transcription:**

The viral reverse transcriptase uses the virus RNA as a template to form complementary DNA.

**4) Integration:**

The newly formed HIV DNA moves to the cell nucleus, where viral integrase mediates its integration into the host cell DNA. Integrated HIV DNA is called 'provirus'.

Therefore, HIV has 2 genomic forms:

- ssRNA (present in the extracellular virus)
- proviral dsDNA (present within the cell)

**5) Transcription:**

HIV mRNA is transcribed from the proviral DNA.

**6) Translation:**

HIV mRNA is transported from the cell nucleus to the cytoplasm, where large viral polyproteins are made using HIV mRNA as a template.

**7) Assembly:**

Assembly of new virus particles takes place at the membrane of the host cell giving an immature virion.

**8) Maturation and Release:**

- The HIV protease cleaves the polyproteins to produce the essential structural proteins and the 3 enzymes mentioned above. This cleavage process is necessary to give the mature infectious virion.
- Release occurs by budding through the cell membrane.

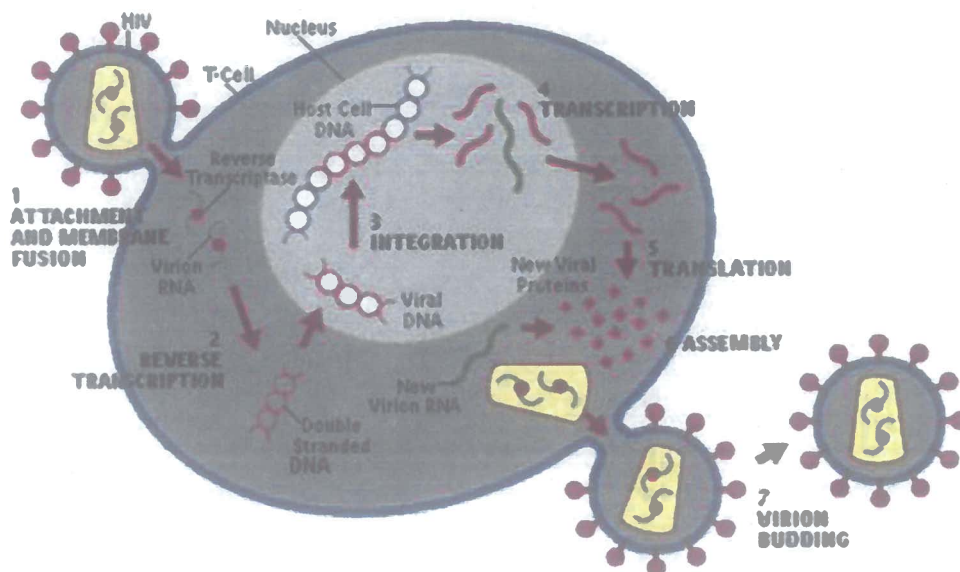


Fig. (10): HIV replication

## Clinical findings

The clinical picture of HIV infection can be divided into 3 stages:

### A) Acute (early) stage:

- After an incubation period of 2-4 weeks, HIV-infected individuals suffer an acute flue-like or infectious mononucleosis-like illness (**acute retroviral syndrome**).
- The most common symptoms are fever, maculopapular rash, oral ulcers, lymphadenopathy, sore throat and malaise.
- During this phase, the blood contains many viral particles that spread throughout the body (particularly lymphoid organs).
- The number of CD4 T cells is usually normal.
- This symptomatic phase lasts for 7-10 days, after which the cytotoxic T cells (the main immune response to HIV) and antibodies dramatically reduce HIV levels. This induced immune response succeeds in controlling, but not eliminating, the virus.

### B) Latent (middle) stage:

- Acute infection is followed by an extended period of **clinical latency**.
- During this phase a large amount of HIV particles is being produced by lymph node cells but remains sequestered within the lymph nodes; i.e. during clinical latency the virus itself does not enter a latent state.
- The patient is usually asymptomatic and viraemia is low or absent.
- The duration of this period (which may extend up to 10 years) depends on several factors including the virus type, immune response and use of antiretroviral therapy.
- Clinical latency is actually attributed to the ability of HIV to evade the aggressive immune response through:
  - a) High rate of viral mutation (especially *env* gene).
  - b) Integration of the virus in the chromosome of infected cells shielded from recognition by the immune system.
  - c) Downregulation of MHC I expression by the virus preventing recognition of infected cells by cytotoxic T cells.
  - d) Loss of CD4 T cell responses which leads to impaired functions of other cells of the immune system.

### C) Immunodeficiency (late) stage (Fig. 11):

- Viral replication and gradual depletion of CD4 T cells continue until finally, some years after initial infection, full-blown **AIDS** develops. This occurs when CD4 T cell count falls below  $200/\text{mm}^3$  (normal count:  $800\text{-}1200/\text{mm}^3$ ).
- The infected person becomes particularly susceptible to opportunistic infections, the most important of which include:
  - Fungal infections: *Pneumocystis jiroveci* (pneumonia)  
*Cryptococcus neoformans* (meningitis)  
*Candida albicans* (oral thrush)
  - Bacterial infections: *M. avium-intracellulare*  
*M. tuberculosis*
  - Viral infections: CMV, HSV, VZV



- A number of malignancies commonly arise in AIDS patients, e.g., Kaposi sarcoma and certain lymphomas. These develop as a result of immunodeficiency and not the virus itself (Fig. 12).
- Patients suffer debilitating weight loss, diarrhoea and neurologic manifestations.
- Infections are the major cause of death in AIDS patients

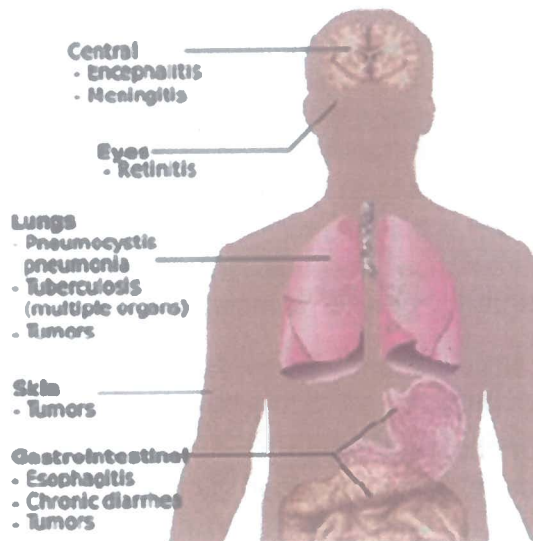


Fig. (11): Symptoms of AIDS



Fig. (12): Kaposi's sarcoma

## Laboratory diagnosis

### 1) Serologic diagnosis:

- **Presumptive diagnosis** of HIV infection can be made by the detection of antibodies by ELISA, using whole virus lysate as antigen. False positive results may occur in certain conditions, e.g. SLE and syphilis.
- **Definitive diagnosis** is done by Western blot technique which is more specific as it detects specific antibodies against certain viral antigens, e.g. gp41 and p24 (Fig. 13).
- Serologic tests are not useful in the diagnosis of the following cases:
  - a) during the acute stage, as antibodies to HIV are not detectable except 3-4 weeks after infection ('window' period),
  - b) newborns of infected mothers due to presence of passively acquired maternal IgG (commercial tests for HIV-specific IgM are not yet available). In such cases, other diagnostic methods should be applied.

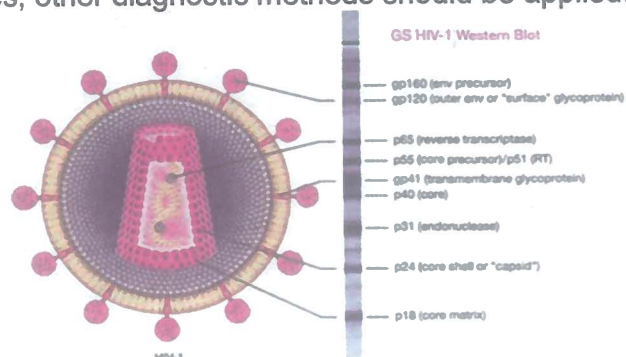


Fig. (13): HIV western blot strip

**2) Molecular techniques for detection of viral nucleic acid:**

These are sensitive and specific techniques.

- PCR can be used to detect proviral DNA within infected cells (qualitative test).
- RT PCR can be used to measure the amount of HIV RNA in plasma to determine viral load (quantitative test).

**3) Antigen detection:**

- p24 antigen is an **early marker** of infection.
- Its presence indicates viral replication. It appears in patient's serum soon after infection and becomes undetectable after development of antibodies which complex with it. Its reappearance late in infection indicates a poor prognosis.
- Detection of p24 antigen by ELISA is now used for routine screening in blood centers to detect HIV during the 'window' period.

**4) Virus isolation:**

HIV can be grown in culture from clinical specimens, but this procedure is available only at a few medical centers.

**5) CD4 count:**

The diagnosis of AIDS can be established by the finding of a CD4 count of less than 200 cells/mm<sup>3</sup> and a CD4/CD8 ratio of less than one (normal ratio is 2:1).

**Treatment**

- A number of antiretroviral drugs have been developed that suppress HIV replication, but eradication of the virus still remains impossible. Thus, HIV infection is currently both chronic and incurable. However, the infection should be treated as aggressively and as early as possible to minimize the initial spread of the virus.
- Antiretroviral drugs target different stages in the replication cycle of HIV (Fig. 14):
  1. **Co-receptor blockers:** These interfere with binding of the virus to the chemokine receptors on the susceptible cells.
  2. **Fusion inhibitors:** These new anti-HIV drugs bind to gp41 preventing fusion of the viral envelope to lymphocytes.
  3. **Reverse transcription inhibitors** (e.g. the nucleoside analogues lamivudine and azidothymidine): These interfere with synthesis of proviral DNA.
  4. **Integrase inhibitors:** These prevent integration of the proviral DNA into the host's genome.
  5. **Protease inhibitors** (e.g. ritonavir and indinavir). These interfere with cleavage of proviral polyproteins during budding resulting in the generation of immature non-infectious viral particles. Protease inhibitors are the most potent antiretroviral drugs
- Currently, HIV infection is treated with a combination of 2 reverse transcription inhibitors and a protease inhibitor. This combination is known as **HAART** (highly active antiretroviral therapy).
- The use of combination regimen limits the possibility of emergence of resistant mutants and permits less toxic doses to be administered.
- Monitoring anti-HIV therapy is done by determination of:
  - a) viral load ( by RT PCR).
  - b) CD4 count.

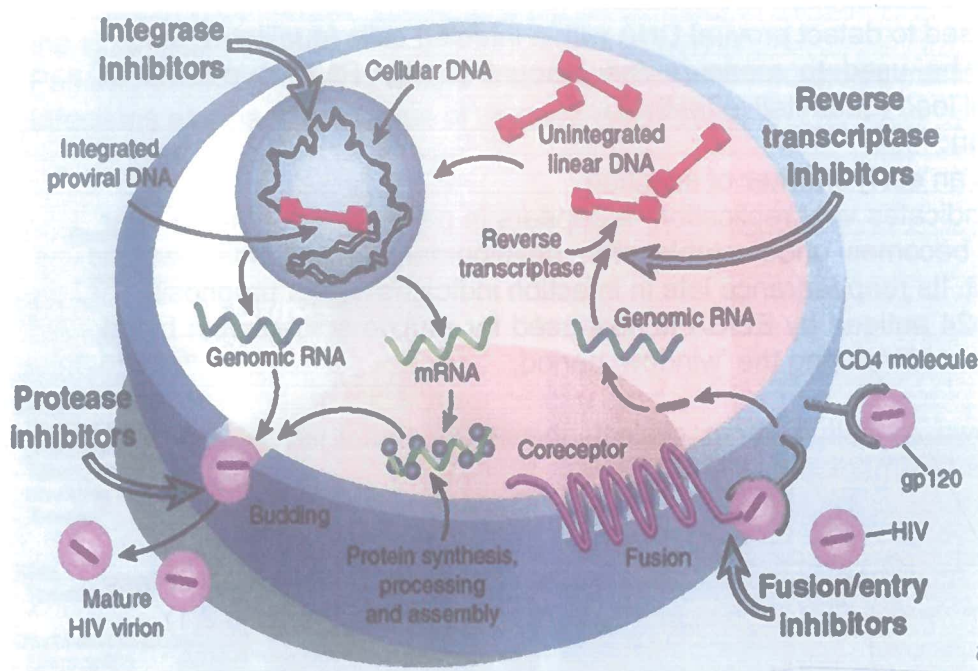


Fig. (14): HIV replication with sites of action of antiviral drugs

### Prevention and Control

- 1) **No vaccine** for human use is available. Trials are being carried out in animal models (monkeys) using a vaccine containing gp120. Antibodies against gp120 neutralize the infectivity of HIV; but the rapid appearance of gp120 genetic variants makes the production of an effective vaccine difficult.
- 2) Prevention depends on **avoiding exposure to the virus**:
  - In the community: through education and awareness of the public regarding modes of transmission.
  - In the healthcare setting: through applying the infection control practices (see Chapter 21).
- 3) In case of exposure to the virus, **post-exposure chemoprophylaxis** should be started within 2 hours using 2 or 3 antiretroviral drugs for 28 days.
- 4) To prevent **vertical transmission** screening of pregnant women for HIV should be done especially among high risk populations. In case of infected women the following measures should be applied:
  - Treatment of infected mothers during pregnancy.
  - Delivery by cesarean section rather than vaginal delivery.
  - Treatment of neonates by effective antiretroviral drugs.
  - No breast feeding.



**MCQs:**

- 1- Which statement regarding HIV is **TRUE**?
  - a- Macrophages can act as reservoir for HIV.
  - b- Highly active antiretroviral therapy is very effective in eradicating HIV infection.
  - c- Neonates born to infected mothers should be tested serologically to establish HIV infection.
  - d- Before entry into a susceptible cell, HIV is called "provirus".
  - e- HIV is a non-enveloped virus.
  
- 2- Which of the following is a receptor for the gp 120 envelope protein of HIV?
  - a- CD3
  - b- CD4
  - c- CD8
  - d- CD28
  - e- CD40
  
- 3- During the acute retroviral syndrome:
  - a- CD4 T cell count falls below  $200/\text{mm}^3$ .
  - b- Patients become vulnerable to opportunistic infection.
  - c- HIV infected patients suffer an acute flu-like illness.
  - d- Patients suffer infections of the lung.
  - e- Patients develop cancers such as Kaposi's sarcoma.
  
- 4- Diagnosis of HIV infection usually starts with one of the following tests:
  - a- ELISA for detection of antibody
  - b- Western blot
  - c- Virus isolation
  - d- PCR to detect proviral DNA in infected cells
  - e- RT PCR to measure amount of HIV RNA in plasma

## ORTHOMYXOVIRUSES

### ILOs:

**By the end of this chapter, the student should be able to:**

- Identify and illustrate the structure of influenza virus
- List the classification and antigenic types of influenza viruses
- Identify the different mechanisms responsible for antigenic changes of the influenza viruses
- Recognize and explain the mechanism of gene reassortment
- Identify the pathogenesis and clinical picture of influenza virus infection
- Summarize the diagnosis of influenza virus infection
- State the treatment of influenza virus infection
- Assess methods used for the prevention of influenza virus infection

The term “myxo” refers to the ability of these viruses to interact with mucins. Influenza viruses are the only members of the orthomyxovirus family. They were so called, because they were thought that the patient is “influenced” by a dark power.

### Influenza Viruses

Influenza viruses are enveloped ssRNA viruses.

#### Structure (Fig. 15)

- The genome is composed of 8 distinct segments of ssRNA.
- Each segment is complexed with nucleoprotein forming a nucleocapsid.
- The nucleocapsids (ribonucleoproteins) are enclosed in an envelope from which 2 types of glycoprotein spikes project:
  - Haemagglutinin (HA): which mediates binding to cell surface receptors to initiate infection.
  - Neuraminidase (NA): which cleaves neuraminic acid on the cell surface to release the progeny virus from the infected cells. It also degrades the protective mucous layer of the respiratory tract helping spread of the virus.

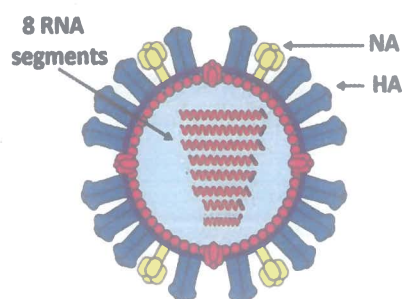


Fig. (15): Structure of Influenza virus.

## Classification and antigenic types

Influenza viruses have a variety of structural proteins that classify them into types and subtypes:

- The internal ribonucleoprotein is the antigen that classifies influenza viruses into 3 types:
  - Type A causes influenza in humans and animals (notably birds and pigs).
  - Type B causes influenza in humans only.
  - Type C causes minor respiratory disease. It is not considered a significant human pathogen.
- Only influenza A virus is further classified into subtypes based upon HA and NA antigens. There are 16 different HA antigens and 9 different NA antigens. Subtypes of influenza A virus are, therefore, designated by the unique combinations of HA and NA antigens, e.g.,:
  - Influenza A/H1N1, the causative agent of swine flu,
  - Influenza A/H5N1, the causative agent of avian flu.
- Antibody against HA neutralizes the infectivity of the virus and prevents disease, whereas antibody against NA does not neutralize infectivity but does reduce disease severity perhaps by decreasing the amount of virus released from the infected cell.

## Antigenic change of the influenza viruses (Fig. 16)

Influenza viruses show marked antigenic variations specifically in HA and NA proteins. This will result in the emergence of new variants of the virus. Individuals who were previously infected with, and hence are immune to, the old variant are thus susceptible to the new variant.

Two different mechanisms are responsible for these antigenic changes:

### 1. Antigenic drift

- This refers to **minor** antigenic changes in HA and NA proteins which generate strains that retain a degree of antigenic relationship with the currently prevailing strains.
- It is the result of mutations in viral RNA.
- Antibodies produced against the previous variant of the virus can still cross-react with the new one and, therefore, most of the population have some level of immunity. As a result, the new variant may cause an epidemic that is relatively mild.
- It occurs each year.
- Antigenic drift may result in epidemics.
- It may occur in all types of influenza viruses (A, B and C).

### 2. Antigenic shift:

- This refers to **major** antigenic changes in HA and NA proteins which generate strains that show no antigenic relationship with the currently prevailing strains.
- It is the result of gene reassortment (see below).
- The new strain is not recognized by antibody directed against the previous variant, so that people are highly susceptible to the new virus, resulting in severe infection.
- It occurs less frequently, perhaps every 10 years.
- Antigenic shift may result in epidemics or pandemics.
- It occurs only in type A influenza virus.



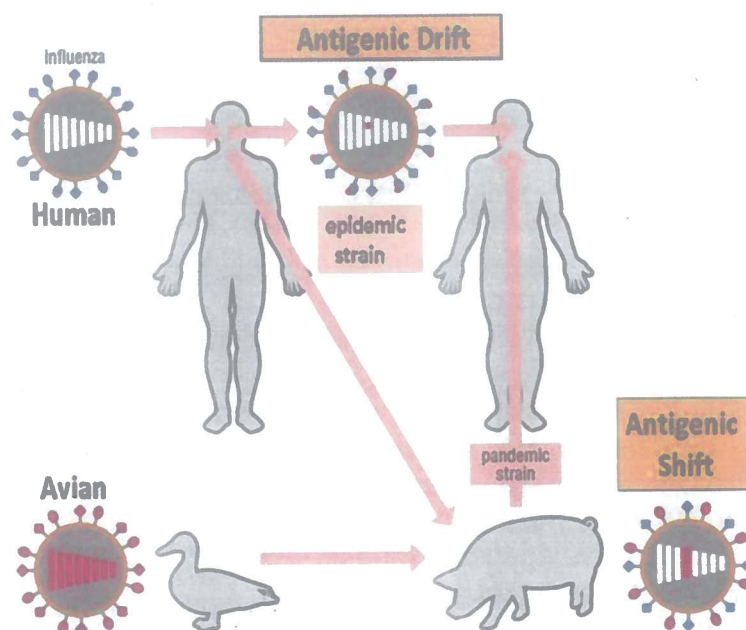


Fig. (16): Antigenic variation of influenza virus

### Gene reassortment

- Because the influenza virus genome is segmented, genetic reassortment can occur when a host cell is infected simultaneously with two different strains of influenza A viruses (e.g., avian and human strains).
- During viral replication, RNA segments can be reassorted (exchanged) to give a progeny virus with novel combination of HA and NA. In this way, new viruses can be generated that differ from both parent strains.
- Pigs play a major role in genetic reassortment, as their cells have receptors for both avian and human influenza strains and can be co-infected by more than one strain. The reassortant virus then has the potential to spread among humans, birds and pigs.
- Gene reassortment does not occur in type B influenza viruses as they have no animal host (i.e., no source for new RNA segments).

### Pathogenesis and clinical picture

- Influenza virus is transmitted by droplets and hand contact with contaminated surfaces, where the virus can survive some hours in dried mucus particularly in cold environment.
- After the virus has been inhaled, the NA degrades the protective mucous layer, allowing the virus to gain access to the cells of the respiratory tract and attach to them by HA.
- Infection results in destruction of superficial mucosa. Viraemia is rare.
- After an incubation period of 1-2 days, the patient suffers from chills, malaise, fever, muscle pain (myalgia) and respiratory symptoms as rhinitis, pharyngitis ... etc. The systemic manifestations are due to cytokines circulating in the blood.
- Influenzal damage to the respiratory epithelium predisposes to secondary bacterial pneumonia (esp. with *S. pneumoniae*, *H. influenzae* and *S. aureus*). Pure viral pneumonia is rare.

## Diagnosis

- During influenza epidemics, diagnosis can generally be made clinically.
- Laboratory diagnosis can be done on nasal or throat washings by:
  - Detection of the viral antigens by ELISA or immunofluorescence.
  - Detection of the viral RNA by RT-PCR.
  - Isolation of the virus in tissue culture.

## Treatment

- Amantadine and rimantadine: These drugs inhibit uncoating. They are no longer recommended because of the following:
  1. They are effective only against type A.
  2. Rapid emergence of drug resistant strains.
  3. Adverse side effects.
- Oseltamivir "Tamiflu" and zanamivir: These drugs are neuraminidase inhibitors. They are active against types A and B.
- These drugs can reduce the severity of influenza, but only if given within 1-2 days of disease onset.

## Prevention

### 1. Vaccination:

- Vaccination is recommended to those at risk of developing complications from influenza infection, e.g., elderly patients.
- The vaccines used provide protection against both types A and B viruses and should be reformulated annually to contain the circulating antigenic strains.
- There are 2 types of influenza vaccines:
  - a) **Killed (formalin-inactivated) vaccine:**
    - It is given by intramuscular injection.
    - It is not a good immunizing vaccine because it yields little secretory IgA.
  - b) **Live attenuated vaccine:**
    - It is given intranasally.
    - It elicits a good protective immune response mediated by secretory IgA.

### 2. Chemoprophylaxis:

The above mentioned antiviral drugs have also been shown to be effective when used for prophylaxis.

**MCQs:**

- 1- All the following statements regarding influenza virus neuraminidase (NA) are correct **EXCEPT**:
  - a- NA helps influenza virus to spread on mucosal surfaces.
  - b- NA allows viral progeny to be released from the infected cells.
  - c- NA projects from the viral envelope.
  - d- NA is found in type A influenza virus only.
  - e- NA is a glycoprotein.
  
- 2- Regarding antigenic shift of influenza virus all the following are true **EXCEPT**:
  - a- It occurs in type A virus only.
  - b- Strains generated by antigenic shift can cause pandemics.
  - c- The new strains generated are serologically similar to the parent strain.
  - d- It is caused by a major change in surface antigens.
  - e- Gene reassortment is responsible for appearance of the new antigens.
  
- 3- **True or false:**
  - a- The genome of influenza A virus is composed of 8 segments of ssRNA.
  - b- Influenza B virus causes infection in humans and animals.
  - c- Live attenuated influenza vaccine is given intranasally.
  - d- The systemic manifestations during influenza infection are due to viraemia.
  - e- Neuraminidase inhibitors are active against types A and B.
  - f- Formalin-inactivated influenza vaccine elicits a good protective response.



## PARAMYXOVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- Identify members of the family *Paramyxoviridae*
- Identify the pathogenesis and clinical findings of parainfluenza viruses
- Identify the pathogenesis and clinical findings of measles virus
- Summarize the prevention of measles
- Identify the pathogenesis and clinical findings of mumps virus
- Summarize the prevention of mumps
- Identify the pathogenesis and clinical findings of respiratory syncytial virus (RSV) and human metapneumovirus infection

- Paramyxoviruses are enveloped ssRNA viruses. Unlike orthomyxoviruses, their genome is non-segmented.
- They include parainfluenza viruses, measles virus, mumps virus, respiratory syncytial virus and human metapneumovirus.

## Parainfluenza Viruses

- Parainfluenza viruses are transmitted via respiratory droplets.
- Incubation period is 2-6 days
- They cause upper and lower respiratory tract disease without viraemia, particularly in infants and young children.
- Infections may include pharyngitis, croup (laryngotracheobronchitis), bronchiolitis and pneumonia.

## Measles (Rubeola) Virus

- Measles is an acute highly infectious disease that occurs primarily in childhood.
- The virus has only one stable antigenic type.

### Pathogenesis and clinical findings

- Man is the only natural host for the virus.
- Transmission is by **aerosol** (airborne).
- The virus replicates locally in the mucosa and regional lymph nodes of the upper respiratory tract followed by viraemia and localization of the virus in skin and mucous membranes.
- Incubation period is 1-2 weeks.
- Clinically, measles begins with prodromal phase of fever associated with cough, coryza (runny nose) and conjunctivitis, often referred to as the “3 Cs” (Fig. 17).



Fig. (17): Manifestations of measles

- 2-3 days later, specific diagnostic signs develop:
  - Koplik's spots (small white spots on bright red buccal mucosa) (Fig. 18)
  - Characteristic maculopapular skin rash beginning at the head and proceeding gradually down the body (Fig. 19)



Fig. (18): Koplik's spots



Fig. (19): Measles rash

- Malnourished children develop more severe disease.
- Complications of measles include:
  - Secondary bacterial infections, especially otitis media and pneumonia.
  - Primary measles virus pneumonia (giant cell pneumonia).
  - Encephalitis which occurs in about 1 in 1000 (mortality rate 10%). This is considered an autoimmune disease.
  - Subacute sclerosing panencephalitis (SSPE): This is a rare fatal disease of CNS that may develop years after apparent recovery. The virus persists in brain and acts as a slow virus.
  - Abortion, premature labour or stillbirth may occur in pregnant women.

### Prevention

- Measles vaccine: This is a living attenuated vaccine that is available in combination with 2 other living attenuated virus vaccines, mumps and rubella (**MMR vaccine**). It is given subcutaneously in two doses, the first at 15 months and the second at school age (4-6 years).
- Immunity is life-long because:
  - measles virus has only one stable antigenic type,
  - there is a stage of viraemia, allowing circulating antibodies (IgM and IgG) to neutralize the virus.

### Mumps Virus

- Mumps is an acute contagious disease of children.
- The virus has only one stable antigenic type.

### Pathogenesis and clinical findings

- Man is the only host for mumps virus.
- Transmission is by droplets.
- The virus replicates locally in the respiratory mucosa and regional lymph nodes, followed by viraemia and localization of the virus in salivary glands especially the parotid glands. The virus may also disseminate into testes, pancreas and brain.
- Incubation period is 2-3 weeks.
- The disease manifests as fever followed by painful non-suppurative swelling of one or both parotid glands (**parotitis**) (Fig. 20).



Fig. (20): Mumps



- Complications of mumps include:
  - Orchitis in post-pubertal males which, if bilateral, can result in sterility.
  - Pancreatitis
  - Meningitis
- At least 1/3 of infections are asymptomatic.

### Prevention

- **MMR vaccine:** (see above).
- Immunity is life-lasting immunity. This is because:
  - only one stable antigenic type exists,
  - there is a stage of viraemia, allowing circulating antibodies (IgM and IgG) to neutralize the virus.

## Respiratory Syncytial Virus (RSV)

- RSV is **the most** important cause of lower respiratory tract disease in infants.
- It is so called due to its ability to cause fusion of host cells forming multinucleated giant cells (**syncytium**). This can be seen as a characteristic CPE in cell culture.
- Humans are the natural host of RSV.
- Transmission is by respiratory droplets or contaminated hands.
- The disease ranges from common cold-like illness in adults to febrile bronchiolitis, bronchitis, and pneumonia in infants and young children.
- It is not associated with viraemia.
- The only specific treatment of RSV infection is ribavirin administered by aerosol.
- Hand washing is the major preventive measure because no vaccine is available.

## Human Metapneumovirus (hMPV)

- hMPV is the second major cause of lower respiratory tract infection in infants and young children.
- It is similar to RSV regarding mode of transmission, diseases and prevention.

**MCQs:**

- 1- **A paramyxovirus that causes the syndrome known as croup is:**
  - a- Adenovirus
  - b- Influenza virus
  - c- Measles virus
  - d- Parainfluenza virus
  - e- Respiratory syncytial virus
  
- 2- **A 3-year-old child presents at the physician's office with symptoms of cough, coryza, conjunctivitis, low-grade fever, and Koplik's spots. The causative agent of this disease belongs to which group of viruses?**
  - a- Adenovirus
  - b- Herpesvirus
  - c- Picornavirus
  - d- Orthomyxovirus
  - e- Paramyxovirus
  
- 3- **Which one of the following best characterizes the vaccine for measles?**
  - a- Toxoid
  - b- Inactivated virus vaccine
  - c- Killed virus vaccine
  - d- Live attenuated virus vaccine
  - e- Recombinant viral vaccine
  
- 4- **True or false:**
  - a- Respiratory syncytial virus is the leading cause of lower respiratory tract infection in infants.
  - b- Human metapneumovirus is closely related to respiratory syncytial virus.
  - c- The genome of mumps virus is single-stranded segmented RNA.

## TOGAVIRUSES

### *ILOs:*

**By the end of this chapter, the student should be able to:**

- State the properties of rubella virus
  - State the pathogenesis and clinical features of rubella
  - Identify the serious complications of rubella if acquired during pregnancy
  - Summarize the laboratory diagnosis of rubella viral infection
  - Identify the prevention and control of rubella virus
  - Identify rubella vaccine, its nature, mode and schedule of administration
- Togaviruses are enveloped ssRNA viruses.
  - Members of medical importance include:
    - Eastern equine encephalitis virus (discussed later).
    - Western equine encephalitis virus (discussed later).
    - Rubella virus.

### Rubella Virus

- It is of only one antigenic type.
- It is the cause of **rubella (German measles)** which is mainly a childhood disease.

### Pathogenesis and clinical findings

- Man is the only natural host of rubella virus.
- Transmission is by droplets.
- The virus multiplies in the respiratory mucosa and local lymph nodes. From there, it spreads via the blood to the internal organs and skin.
- After an incubation period of 2-3 weeks, fever develops followed by maculopapular rash which starts on the face and spreads downwards (Fig. 21). The rash typically lasts 3 days.
- Posterior auricular lymphadenopathy is characteristic.
- 25% of the cases may be asymptomatic.



**Fig. (21): Rubella rash**



### Infection during pregnancy

- Although rubella is a mild self-limiting disease, if it is acquired during the first trimester of pregnancy it may be transmitted transplacentally resulting in serious complications.
- There may be abortion, intrauterine foetal death, or congenital malformations of the baby such as mental retardation, deafness, heart anomalies, microcephaly, cataract and blindness. This is called **congenital rubella syndrome** (Fig. 22).

### Laboratory diagnosis

- Laboratory diagnosis is usually not necessary except in pregnant females. Detection of rubella antibodies by ELISA, either IgM or a rising titre of IgG, denotes recent infection and therapeutic abortion may be indicated.
- Detection of IgM anti-rubella antibodies in a newborn confirms intrauterine infection.

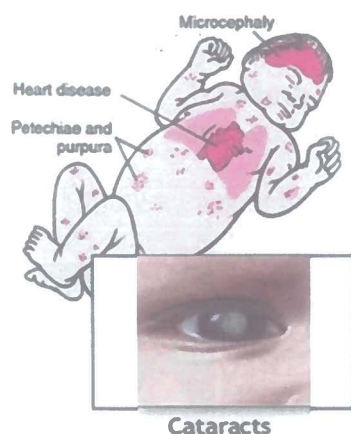


Fig. (22): Congenital Rubella Syndrome

### Prevention and control

- Rubella can be prevented by **MMR** vaccine (see above).
- For the prevention of congenital rubella syndrome, a booster dose is recommended for prepubertal girls and for mothers in the immediate postpartum period.
- It is advisable to avoid vaccination during pregnancy.
- Conception should be delayed for at least 3 months following vaccination.

### MCQs:

#### 1- Rubella infection can be prevented by

- a- A heat inactivated (killed) vaccine
- b- A formalin inactivated toxoid
- c- A recombinant vaccine
- d- A split vaccine
- e- A living attenuated vaccine

## CORONAVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- List the properties of coronaviruses
- Identify diseases caused by human coronaviruses (CoVs)
- Identify the clinical presentations caused by SARS-CoV

- Coronaviruses are enveloped, ssRNA viruses.
- The family received the name “corona” because of the crown-like appearance of the surface projections (Fig. 23).

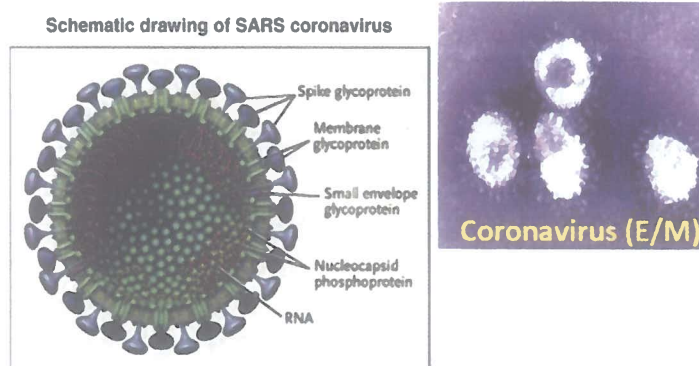


Fig.(23): Structure of coronavirus

### Mode of transmission

- Inhalation of respiratory droplets
- Contact of the hands with contaminated surfaces and then touching eyes, nose or mouth.

### Diseases

#### 1- Common Cold:

- This is a mild upper respiratory tract illness.
- Coronaviruses are the second most common cause of common cold following the rhinoviruses.

**2- Severe Acute Respiratory Syndrome (SARS):**

- SARS is caused by a newly emerged member of coronaviruses, the SARS coronavirus (SARS-CoV).
- It is a severe atypical pneumonia characterized by fever, dry cough and dyspnea.
- Diarrhoea has been a prominent feature of early illness in some cases.
- The incubation period ranges from 2-10 days.
- The mortality rate is about 10%.
- SARS-CoV was first detected in China in 2002 and subsequently spread to many countries. This outbreak subsided, and no new cases have been identified since 2004.

**3- Middle East Respiratory Syndrome (MERS):**

- MERS is a severe acute respiratory illness caused by the recently identified MERS coronavirus (MERS-CoV).
- MERS-CoV was first reported in Saudi Arabia in 2012 and since then it has been reported in other countries mainly in the Middle East.
- The mortality rate is about 35%.

**Diagnosis**

- Diagnosis of common cold is primarily a clinical one.
- SARS and MERS are only suspected if there is prior history of travel to an endemic area. Laboratory confirmation is done by detection of antibodies and by PCR.

**MCQs:**

- 1- One of the following viruses has a characteristic electron microscopic morphology:
  - a- Coronaviruses
  - b- Rubella virus
  - c- Measles virus
  - d- Mumps virus
  - e- Hepatitis A virus
- 2- **True or false:**
  - a- Coronaviruses are DNA viruses.
  - b- The name "corona" is due to the crown-like appearance of the envelope.
  - c- Coronaviruses are the most common cause of "common cold".
  - d- SARS is caused by a member of coronaviruses.
  - e- SARS-CoV can cause Middle East respiratory syndrome.
  - f- Diarrhoea is a prominent feature of SARS in some cases.
  - g- MERS has a higher mortality rate than SARS.
  - h- MMR vaccine can be used to prevent MERS and SARS.



## RHABDOVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- Describe the characteristic structure of rabies virus
  - Identify the main reservoirs of rabies virus
  - Identify the transmission of rabies infection
  - Identify pathogenesis and clinical manifestations of rabies infection
  - List laboratory diagnosis of rabies in humans and animals
  - List post-exposure prophylaxis of rabies
  - Identify rabies vaccines
  - Identify pre-exposure prophylaxis of rabies
- Rhabdoviruses are enveloped bullet-shaped ssRNA viruses with helical symmetry (Fig. 24).
  - Rabies virus is the only medically important member of the rhabdovirus family.

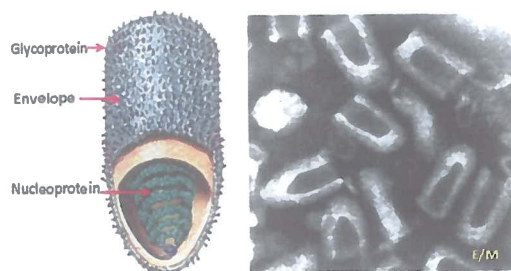


Fig. (24 ): Structure of Rabdoviruses (Rabies virus)

### Rabies Virus

- Rabies virus is a neurotropic virus. It causes rabies, which is an acute fatal encephalomyelitis.
- Rabies is a zoonotic viral disease which infects domestic and wild animals.

### Mode of transmission

- The virus can infect all mammals but important sources of infection for humans include dogs, cats, wolves, foxes and bats.
- Rodents and rabbits do not transmit rabies.
- The virus is excreted in the saliva of infected animals.
- Transmission to man may occur following:
  - bite of a rabid animal
  - non-bite exposure to the virus: Salivary contamination of skin abrasions or wounds, and exposure to aerosols of bat secretions.

### Pathogenesis (Fig. 25)

- The virus replicates at the site of the bite and then travels along the peripheral nerves to the CNS (no viraemia).
- The virus multiplies in the nerve cells forming characteristic intra-cytoplasmic inclusion bodies known as “**Negri bodies**”.
- The virus then travels down the peripheral nerves to the salivary glands to be excreted in the saliva.

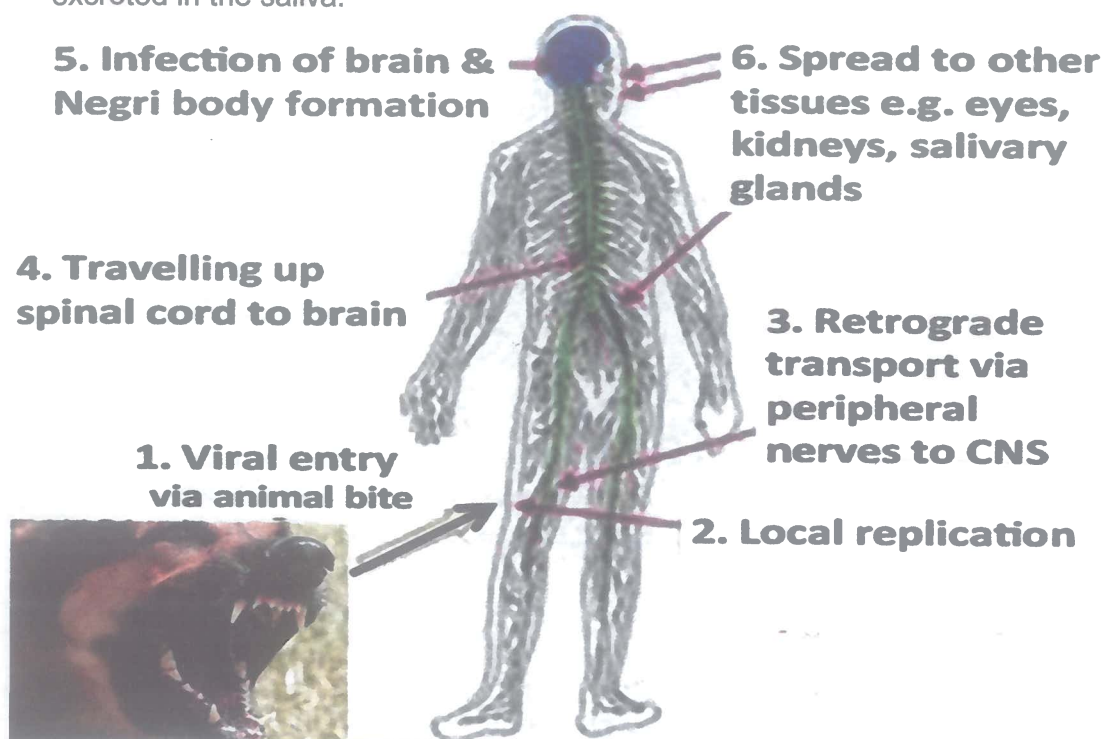


Fig. (25): Pathogenesis of Rabies

### Incubation period

- The incubation period is extremely variable ranging from 2-16 weeks or even longer.
- The duration of incubation period depends on:
  - Distance of the site of the bite from the CNS (the further the bite from the CNS, the longer the incubation period).
  - The severity of the bite and the amount of the inoculated virus.
  - The immune status of the host.

### Clinical manifestations

- Once symptoms of the disease develop, rabies is almost always fatal to both animals and humans.
- Clinically, the patient exhibits a prodrome of non-specific symptoms such as fever, and changes in sensation at the bite site (paraesthesia).
- The disease then progresses to fatal encephalitis manifesting as hallucinations, seizures and increased salivation.
- Many patients show the classic rabid sign of hydrophobia which is avoidance of drinking water due to painful spasm of the pharyngeal muscles on swallowing.
- Death occurs as a result of cardiac or respiratory arrest.

## Laboratory diagnosis

During its transport within the nerve, the virus is sequestered from the immune system; therefore, there is no detectable antibody or cell-mediated immune response.

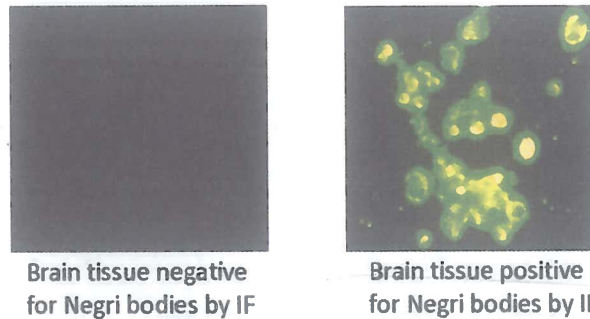
- **Diagnosis in humans:**

Prior to death, diagnosis can be made by detection of viral antigens or nucleic acid in infected saliva or skin biopsies from the back of the neck.

- **Diagnosis in animals:**

Diagnosis is made post-mortem by examination of brain tissue using:

- Direct immunofluorescence or histologic staining to detect Negri bodies (Fig. 26 )
- PCR to detect viral nucleic acid



**Fig. (26): Negri bodies by immunofluorescence**

## Management of rabies

There is no antiviral therapy for a patient with rabies. Fortunately, rabies infection can be aborted by using preventive measures; the long incubation period gives anti-rabies vaccines administered after the bite sufficient time to induce protective immunity.

For prevention of rabies the following should be applied:

- I. **Care of the wound:**

This is done by:

- Thorough cleaning of the wound by soap and water.
- Irrigation of the wound with an antiseptic agent.

- II. **Post-exposure prophylaxis (PEP):**

This includes the following (Table 6):

1. **Human rabies immune globulin (HRIG):**

- HRIG should be administered as early as possible to ensure prompt passive immunization.
- As much as possible of the HRIG dose is infiltrated into the wound and the remainder of the HRIG, if any, is given intramuscularly. HRIG is given to neutralize the virus at the bite site and to give time for the vaccine to stimulate active immunity before the virus reaches the CNS.
- HRIG and rabies vaccine should be given at different sites to prevent neutralization of the virus in the vaccine by the antibody in the HRIG.

2. **Rabies vaccines:**

- The human diploid cell vaccine (HDCV) contains inactivated virus grown in human diploid cells. The vaccine is given in 5 intramuscular doses (on days 0, 3, 7, 14 and 28).



- Vaccines grown in monkey lung cells and chick embryo cells are also available.
- The old nerve tissue vaccines are not recommended as they are less immunogenic, require 23 injections and cause allergic encephalomyelitis.

N.B.: PEP for a previously vaccinated individual involves only 2 doses of anti-rabies vaccine (on days 0 and 3) without the need for HRIG.

### Indications for PEP:

- If the animal is not available, PEP is indicated.
- If the animal is available: The animal should be observed for 10 days:
  - If no symptoms appear within this period the diagnosis of rabies is excluded and PEP is not indicated.
  - If the animal dies or symptoms appear, the animal is sacrificed and brain tissue is examined for rabies. PEP is indicated if the diagnosis of rabies is established.
- After bites with expected short incubation period as in children or bites in the head and neck (even if the animal is available), PEP should be started immediately to be discontinued if the animal proves not to be rabid.
- Bite or non-bite exposure to bats necessitates PEP.

### Control

Pre-exposure vaccination is recommended for persons at risk e.g. veterinarians. It is given in 3 intramuscular doses.

- Vaccination of pets e.g. dogs and cats, and control of stray dogs

**Table (6): Rabies post-exposure prophylaxis schedule**

Vaccination Status	Management	Regimen
Not previously Vaccinated	HRIG	<ul style="list-style-type: none"> <li>- As early as possible</li> <li>- Full dose: around the wound(s)</li> <li>- Remaining volume (if any): IM</li> </ul>
	Vaccine	<ul style="list-style-type: none"> <li>- HDCV: 5 IM doses</li> <li>On days 0, 3, 7, 14, and 28</li> </ul>
Previously vaccinated	Vaccine	<ul style="list-style-type: none"> <li>- HDCV: 2 IM doses</li> <li>On days 0 and 3</li> </ul>

**MCQs:**

- 1- **Which of the following viruses can cause fatal encephalitis?**
  - a- Rabies virus
  - b- Rhinovirus
  - c- Cytomegalovirus
  - d- Respiratory syncytial virus
  - e- Mumps virus
  
- 2- **The presence of Negri inclusion bodies in host cells is characteristic of which of the following?**
  - a- Aseptic meningitis
  - b- Congenital rubella
  - c- Infectious mononucleosis
  - d- Mumps
  - e- Rabies
  
- 3- **Post-exposure prophylaxis for previously vaccinated persons include:**
  - a- Administration of HRIG as early as possible
  - b- Administration of 5 IM doses of rabies vaccine
  - c- Wound care and HRIG
  - d- Wound care and 2 IM doses of rabies vaccine
  - e- No prophylaxis is required in previously vaccinated persons
  
- 4- **True or false:**
  - a- Rabies virus is a neurotropic virus.
  - b- Rabies is a zoonotic disease.
  - c- Rodents can transmit rabies.
  - d- Rabies may be transmitted by non-bite exposure to the virus.
  - e- Negri bodies are intranuclear inclusion bodies.
  - f- Rabies virus reaches the CNS haematogenously.

## ARBOVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- List the properties of arboviruses
- Outline the classification of arboviruses
- Identify the pathogenesis and clinical findings of yellow fever
- State the major epidemiologic cycles of yellow fever
- Summarize the prevention and control of yellow fever
- Identify the vaccine used in the prevention of yellow fever
- Identify the pathogenesis and clinical findings of dengue fever
- Identify the pathogenesis and clinical findings of Zika fever
- Identify the pathogenesis of West Nile encephalitis
- Identify the pathogenesis and clinical findings of Rift Valley fever
- Identify the prevention of Rift Valley fever

- Arboviruses are **arthropod-borne** viruses, i.e. they are transmitted by arthropods.
- It is a collective name for a large group of diverse viruses belonging to **different** families.
- In general, they are named either for the diseases they cause (e.g., yellow fever virus) or for the place where they were first isolated (e.g., West Nile virus).
- Most arboviruses are classified in 3 families, namely:
  - I. *Flaviviridae*.
  - II. *Togaviridae*.
  - III. *Bunyaviridae*.

### I. *Flaviviridae*

- Flaviviruses are enveloped ssRNA viruses.
- Members of medical importance include:
  - Yellow fever virus
  - Dengue fever virus
  - Zika virus
  - West Nile encephalitis virus

N.B.: Hepatitis C virus (previously discussed) is a member of *Flaviviridae* but is not transmitted by arthropods (i.e., not an arbovirus).



## Yellow Fever

Yellow fever is an acute, febrile, haemorrhagic disease caused by yellow fever virus. As the name implies, yellow fever is characterized by jaundice and fever.

### Mode of transmission

Yellow fever virus is transmitted by mosquito bite and occurs in 2 different epidemiologic cycles (Fig. 27):

1. **Jungle yellow fever** is primarily a disease of monkeys; the disease is transmitted from monkey to monkey by *Aedes africanus* mosquito in Africa. Man is infected accidentally during his work in forests (wood cutters, road builders) and carries the infection to urban areas.
2. **Urban yellow fever** involves man to man transmission by *Aedes egypti* which breeds in stagnant water near houses.

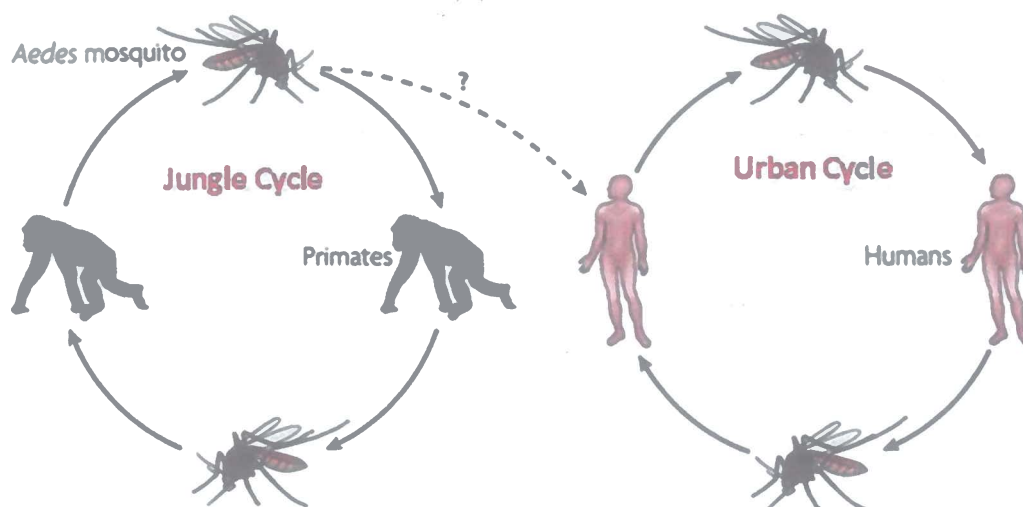


Fig. (27): Yellow fever cycle

### Pathogenesis

- From the site of the bite, the virus reaches the local lymph nodes where it multiplies and then enters the blood stream.
- The virus infects the vascular endothelium, the liver, and kidneys.
- After an incubation period of 3-6 days, there is high fever, jaundice, albuminuria and gastrointestinal haemorrhages ("black vomit").
- The high mortality rate in severe cases is due to liver and kidney failure.

### Prevention

- Mosquito control.
- **17D Vaccine:** It is a live attenuated vaccine given as a single subcutaneous injection. It is very effective and protection persists for 10 years. It is given to travelers to endemic areas.

## Dengue Fever

- Dengue fever virus has the same mode of transmission as yellow fever (Fig. 28).
- The disease occurs in 2 clinical forms:
  - **Classic dengue** ("breakbone fever") which begins suddenly with influenza-like manifestations with severe pains in the bones and muscles. Complete recovery is the rule.
  - **Dengue haemorrhagic fever** which is a much more severe disease affecting mainly children. The acute onset of fever is accompanied by gastrointestinal haemorrhages, renal involvement and shock with case fatality rate of 50%.

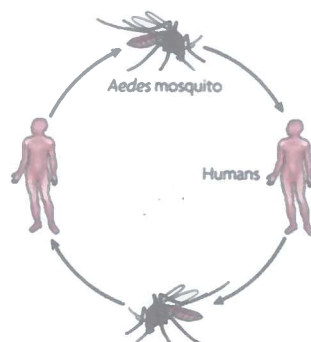


Fig. (28): Dengue fever virus transmission

## Zika Fever

- Zika virus is primarily spread by the bite of infected mosquitos (*Aedes aegypti*) like yellow fever and dengue fever viruses. However, sexual and blood-borne transmission of Zika virus has also been documented.
- After inoculation of the virus into human skin, the virus spreads to lymph nodes and then enters the bloodstream.
- Most cases have no symptoms, but when present they are usually mild and may include fever, red eyes, joint pain, headache and a maculopapular rash. Symptoms generally last less than seven days (Fig. 29).

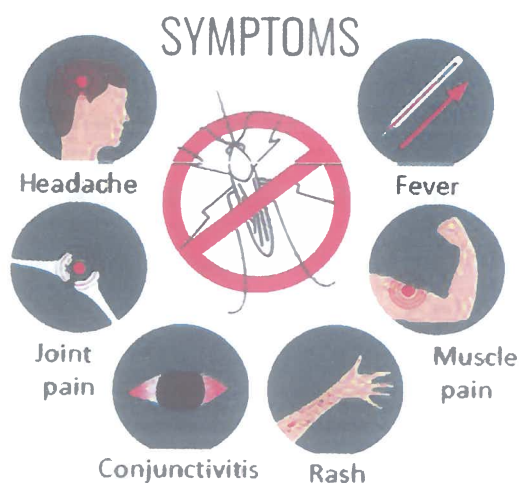


Fig. (29): Symptoms of Zika virus infection

## Zika Virus Microcephaly



Fig. (30): Microcephaly caused by Zika virus infection

- Infection during pregnancy may cause **microcephaly** and other foetal brain malformations (Fig. 30)
- Infection in adults has been linked to Guillain–Barré syndrome.
- There is no specific treatment and prevention is by protection against mosquito bites.

### West Nile Encephalitis

- West Nile encephalitis virus is endemic in Africa.
- Wild birds are the main reservoir of this virus which is transmitted by mosquito bite (Fig. 31)
- The virus multiplies in the reticuloendothelial cells and is then released into the blood. Viraemia coincides with the onset of fever. The virus then reaches the CNS causing meningitis or meningoencephalitis.
- Inapparent infections are common.

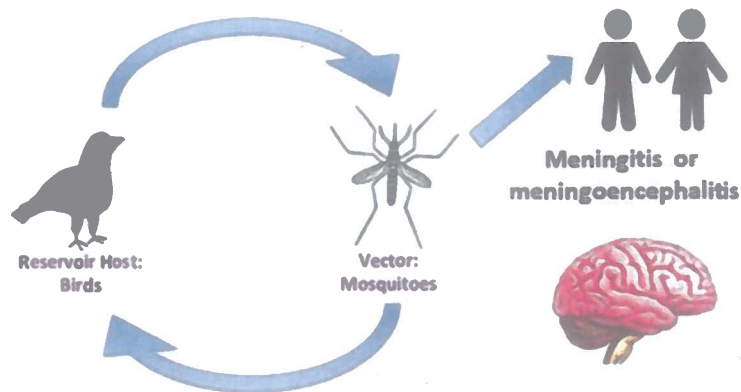


Fig. (31): Transmission cycle of West Nile encephalitis virus

## II. Togaviridae

- Togaviruses are enveloped ssRNA viruses.
- Members of medical importance include:
  - Eastern equine encephalitis virus.
  - Western equine encephalitis virus.
  - Rubella virus.

N.B.: Rubella virus (previously discussed) is a member of *Togaviridae*, but is not transmitted by arthropods (i.e., not an arbovirus).

## III. Bunyaviridae

- Bunyaviruses are enveloped ssRNA viruses.
- Members of medical importance include:
  - California encephalitis virus.
  - Rift Valley fever virus.
  - Hantaan virus (see chapter 10).



## Rift Valley Fever

- Rift Valley fever virus is primarily a pathogen of sheep and domestic animals.
- Man is infected through (Fig. 32):
  - Contact with infected animals,
  - Mosquito bite.
- Disease in humans is usually a mild febrile illness with complete recovery.
- Complications include haemorrhagic fever and encephalitis.
- An outbreak of Rift Valley fever occurred in Egypt during 1977 and caused 600 human deaths.
- A living attenuated vaccine is used for animal immunization.

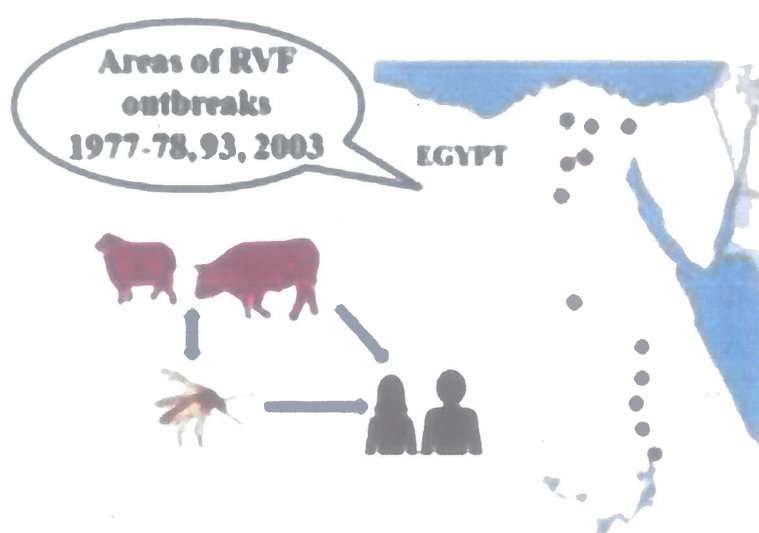


Fig. (32): Transmission and areas of outbreaks of Rift Valley fever

### MCQs:

- 1- The nature of yellow fever vaccine is:
  - a- Recombinant
  - b- Heat inactivated
  - c- Formalin inactivated
  - d- Living attenuated
  - e- Plasma derived
- 2- Zika virus infection:
  - a- Is primarily spread by droplets
  - b- May be transmitted sexually
  - c- Is not associated with viraemia
  - d- Can be prevented by a specific vaccine
  - e- Has a fatal outcome

## ROBOVIRUSES

### **ILOs:**

**By the end of this chapter, the student should be able to:**

- List the properties of roboviruses
  - Outline the classification of roboviruses
  - Recognize the pathogenesis of fever caused by the Hantavirus genus
  - Recognize the clinical findings of fever caused by the Hantavirus genus
  - Recognize Lassa fever virus
- 
- Roboviruses are **rodent-borne** viruses that are transmitted directly from rodents to humans **without arthropod vectors**.
  - Medically important members include:
    - Lassa fever virus which belongs to *Arenaviridae*.
    - Hantaviruses which belong to *Bunyaviridae*.

### **Lassa fever virus**

- **Lassa fever virus** is the most important member of Arenaviruses which are enveloped ssRNA viruses.
- It causes haemorrhagic fever with high mortality rate.
- The virus is shed with rodent excreta, and is transmitted to humans by:
  - inhalation of contaminated aerosol,
  - ingestion of contaminated food,
  - contact with contaminated soil.
- Man-to-man transmission occurs especially in hospitals.

## Hantaviruses

- The most important member of Hantaviruses is **Hantaan virus** which causes Korean haemorrhagic fever.
- They are transmitted to humans by inhalation of aerosols of infected rodent excreta (Fig. 33)
- There is no person-to-person transmission.
- Other members of Hantaviruses cause Hantavirus pulmonary syndrome which is associated with high mortality.

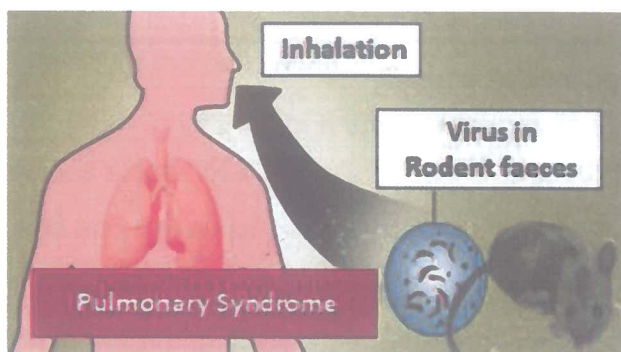


Fig. (33 ): Transmission of Hantaviruses

Table (7): Classification of arboviruses and roboviruses

	Family	Important Viruses
Arboviruses	Flaviviruses	<ul style="list-style-type: none"> <li>- Yellow fever virus</li> <li>- Dengue fever virus</li> <li>- Zika virus</li> <li>- West Nile encephalitis virus</li> </ul>
	Togaviruses	<ul style="list-style-type: none"> <li>- Eastern equine encephalitis virus</li> <li>- Western equine encephalitis virus</li> </ul>
	Bunyaviruses	<ul style="list-style-type: none"> <li>- California encephalitis virus</li> <li>- Rift Valley fever virus</li> </ul>
Roboviruses		- Hantaan virus
	Arenaviruses	- Lassa fever virus

### MCQs:

#### 1- Roboviruses:

- Are transmitted by arthropods
- Have rodents as the main reservoir
- Include Marburg and Ebola viruses
- Belong to the Flaviviridae family
- Do not include Hantavirus



## FILOVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- List the properties of Filoviruses
  - State the pathogenesis of fever caused by Marburg and Ebola viruses
  - Identify the clinical findings of fever caused by Marburg and Ebola viruses
- Filoviruses are enveloped ssRNA viruses.
  - They appear as filaments of varying lengths (Fig. 34).
  - Medically important members include:
    - **Ebola virus**
    - **Marburg virus**
  - Both viruses cause severe haemorrhagic fever characterized by widespread bleeding into the skin, mucous membrane, visceral organs and the gastrointestinal tract (Fig. 35)



Fig. (34): Ebola virus



Fig. (35): Ebola (Haemorrhagic fever)

- Mortality rate is high, and may reach up to 90%.
- Although the natural reservoir of these viruses is unknown, they can be transmitted to humans from infected monkeys or by exposure to blood or other body fluids from an infected patient. Medical staff is especially at risk.
- In 2014, an epidemic of Ebola killed many thousands mostly in the west African countries.

### MCQs:

#### 1- Filoviruses:

- Are transmitted by arthropods
- Appear as filaments of varying lengths
- Include Hantaan and Lassa fever viruses
- Belong to the Flaviviridae family
- Are DNA viruses

## PICORNAVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- List the important genera of Picornaviruses
- Summarize enterovirus groups and serotypes and identify the major diseases caused by them
- State the properties of the poliovirus
- Summarize the pathogenesis and clinical features of poliomyelitis and explain the various stages of infection
- State prevention and control of poliomyelitis
- Compare and contrast Sabin vaccine and Salk vaccine
- State the pathogenesis and clinical significance of rhinoviruses
- Explain the causes of repeated infection of common cold and failure of vaccine production for rhinoviruses

- Picornaviruses (Pico= small, rna = RNA) are small non-enveloped ssRNA viruses.
- Important genera include (Fig. 36):

### 1. *Enterovirus* (Table 8):

- Enteroviruses are transmitted by faeco-oral route.
- They are stable at the low pH of the stomach, and replicate in the GIT without causing diarrhoea.
- They can enter the blood stream and, thereby, spread to various target organs particularly CNS causing aseptic meningitis.

**Table (8): *Enterovirus* groups and major diseases**

Group	Major diseases
<b>Poliovirus</b>	1. Aseptic meningitis 2. Paralytic poliomyelitis
<b>Coxsackieviruses A</b>	1. Aseptic meningitis 2. Herpangina (febrile pharyngitis) 3. Hand, foot and mouth disease (rash on hands and feet, and ulcers in mouth)
<b>Coxsackieviruses B</b>	1. Aseptic meningitis 2. Myocarditis 3. Pleurodynia (stabbing chest pain) 4. Juvenile diabetes mellitus
<b>Echoviruses</b> (Enteric Cytopathogenic Human Orphan Viruses)	1. Aseptic meningitis 2. Fever and rash

N.B.: As new enteroviruses are identified, they are not assigned to one of these groups but are simply given numerical designations (e.g., enterovirus 68, enterovirus 69..... and so on).

2. **Hepatovirus (HAV).**

3. **Rhinovirus.**

4. **Aphthovirus:** It is the causative agent of foot-and-mouth disease that occurs primarily in cattle and sheep. It can be transmitted to humans by contact or ingestion of infected meat.

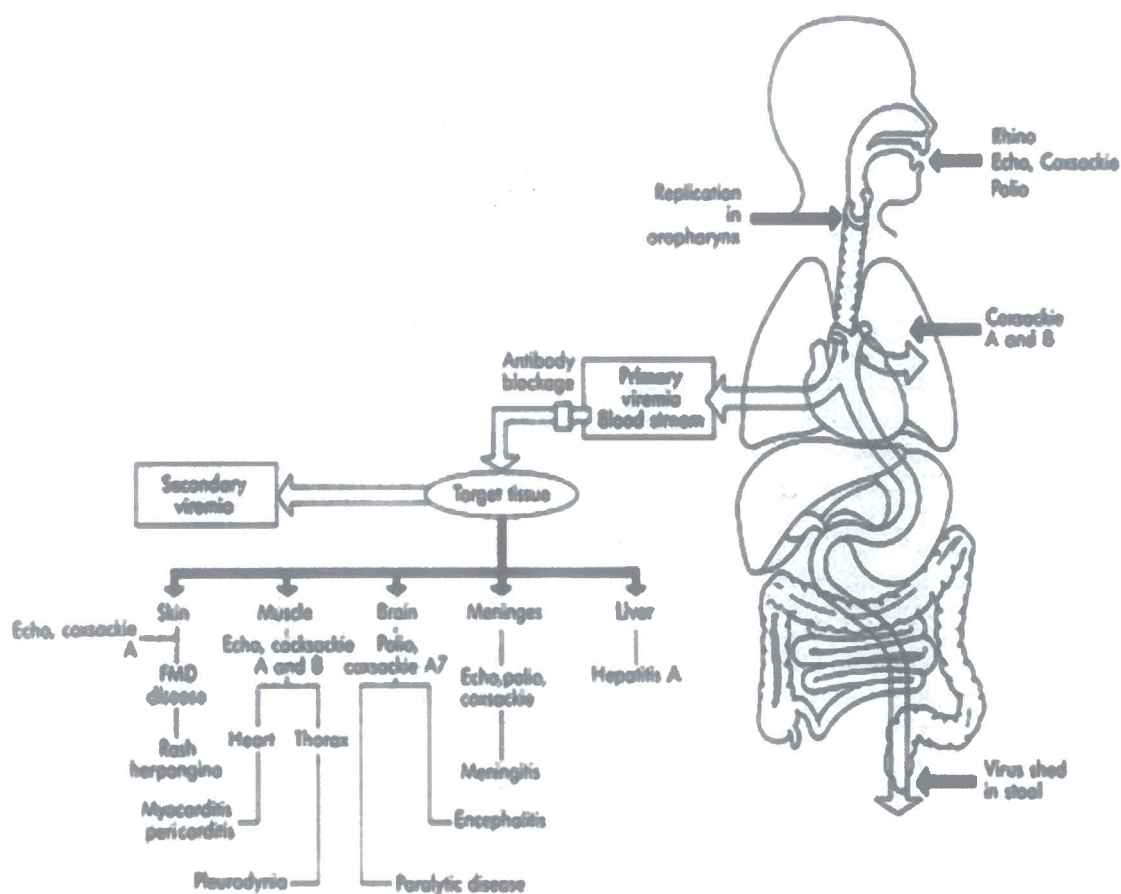


Fig. (36): Picornaviruses

## Poliovirus

### Properties of the virus

1. There are three antigenic types (1, 2 and 3).
2. The virus is inactivated by pasteurization and chlorine.
3. Primates, e.g. human and monkeys are the only susceptible hosts.



## Poliomyelitis

### Pathogenesis and clinical features (Fig. 37)

- Infection occurs by ingestion of food and drinks contaminated by stools from cases or carriers (i.e. faeco-oral).
- Most of the infections are subclinical (asymptomatic), only 1% of the infections result in clinical illness.
- The incubation period is 7-14 days.
- The infection may occur at various stages as follows:
  1. **Inapparent (asymptomatic) infection:** The virus multiplies in the intestinal lymphoid tissues without invading the blood stream or CNS. It is excreted in faeces. At this stage, protective immunity develops against re-infection with the same antigenic type.
  2. **Abortive infection:** It is the most common clinical form. The virus invades the blood stream (viraemia) but not the CNS. Patients present with mild symptoms as fever, malaise, headache, nausea and vomiting.
  3. **Aseptic meningitis (non-paralytic poliomyelitis):** The virus reaches the CNS. The manifestations are headache, neck stiffness and back pain. The condition usually resolves spontaneously.
  4. **Paralytic poliomyelitis:** The virus selectively destroys the lower motor neurons of the spinal cord resulting in flaccid paralysis. This develops in 0.1-1% of infections especially in children. Involvement of the brain stem can lead to life-threatening respiratory paralysis.

N.B.: Faecal carriage of the virus usually occurs for several months (no permanent carriers).

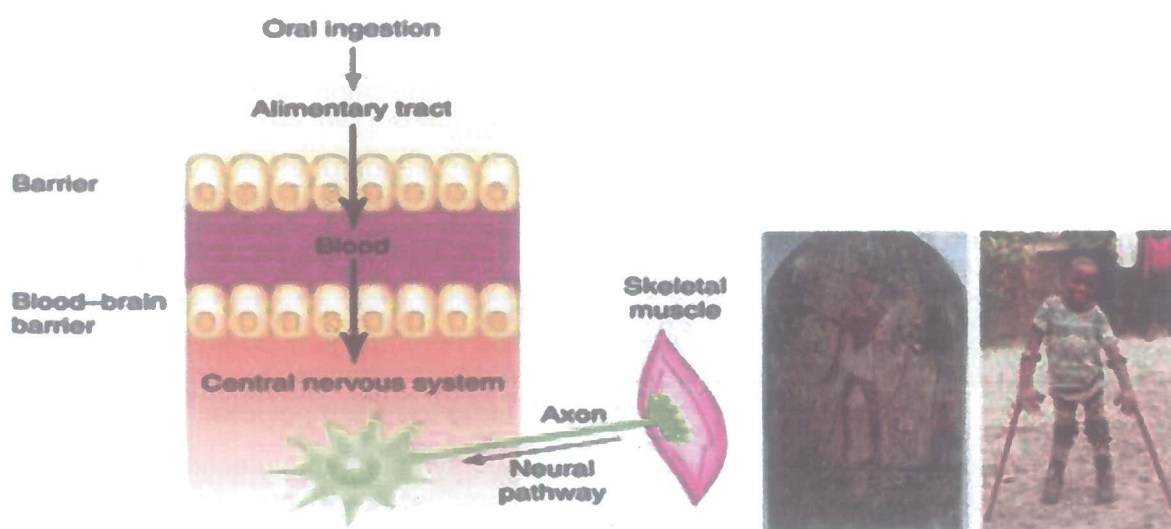


Fig. (37): Pathogenesis of poliomyelitis

## Diagnosis

1. Isolation of the virus from throat, stools or CSF by inoculation on cell cultures derived from human or monkey tissues. The virus causes CPE that can be neutralized by specific antisera.
2. Demonstration of rising antibody titer.

## Prevention

- Vaccination is the only effective method of preventing poliomyelitis.
- There are two effective vaccines containing the three antigenic types:
  - a live attenuated polio-vaccine (Sabin)
  - a formalin-inactivated polio-vaccine (Salk)
- The vaccine is given to infants in three doses at 2, 4 and 6 months. Booster doses are given at 18 months and at school entry.

### The Live attenuated poliovaccine (Sabin vaccine):

#### Advantages:

- It is given orally (oral poliovaccine; **OPV**).
- It provides both local intestinal immunity (by secretory IgA) and systemic immunity (by IgM and IgG). Thus, it prevents all stages of poliovirus infection.
- The attenuated vaccine strains pass with the stools and disseminate among the population, thus, replacing the wild strains. This leads to spread of the immunity in the community.

#### Disadvantages:

- It is contraindicated in pregnancy and in immunosuppressed individuals.
- Infection of the GIT by other enteroviruses can limit replication of the vaccine virus and reduce protection (interference phenomenon).
- It must be kept refrigerated (at 4°C) to prevent heat inactivation of the live virus.
- Rarely, reversion of the attenuated virus to the wild type may occur, resulting in vaccine-associated poliomyelitis.

### The inactivated polio vaccine (IPV) (Salk vaccine)

- It is given by IM injection.
- It provides only systemic immunity (by IgM and IgG), thus, preventing CNS infection.
- It does not provide local GIT immunity; accordingly, asymptomatic infection with the wild poliovirus and its transmission to other persons may occur. This may hinder the efforts for eradication of the wild type.
- Being killed, it has the following **advantages** over the Sabin vaccine:
  - It is safe to be given in pregnancy and immunosuppressed individuals.
  - It does not require refrigeration.
  - It does not revert to the wild type.

## Rhinoviruses

- The rhinovirus group derives its name from the predominant site of replication (the nose).
- The rhinovirus group is the commonest cause of the acute respiratory illness known as the **common cold**.
- Rhinoviruses replicate better at 33°C which explains why they affect primarily the nose and conjunctiva rather than the lower respiratory tract.
- They are acid labile, therefore, they are killed by gastric acidity and cannot infect GIT.
- Over 100 serotypes of rhinoviruses are currently recognized (Fig. 38).

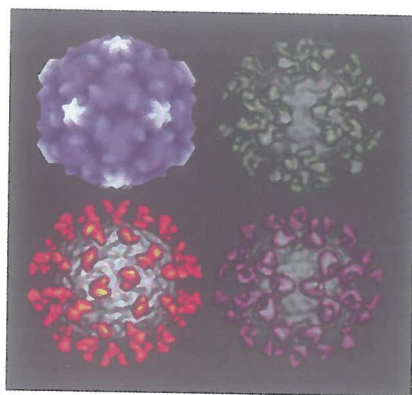


Fig. (38): Rhinoviruses (multiple serotypes)

### Pathogenesis and clinical significance

- Transmission is through droplet and hand-to-hand contact followed by self-inoculation of virus into the nose or conjunctiva.
- Sneezing, nasal discharge, sore throat, cough and headache occur after an incubation period of 2-3 days.
- The virus multiplies locally without blood invasion.
- Secondary bacterial infection may complicate rhinovirus infection, resulting in otitis media, sinusitis, bronchitis or bronchopneumonia especially in children.
- Repeated infection of common cold could be explained by:
  1. Multiplicity of antigenic types. As immunity is serotype-specific, infection with a certain serotype does not protect against other serotypes.
  2. Superficial nature of infection. This results in short term immunity mediated by secretory IgA and interferon. Serum antibodies play no significant role.
  3. Unavailability of vaccines. Vaccine production is not practical because of the large number of serotypes.



**MCQs:**

- 1- Enteroviruses are characterized by all of the following **EXCEPT**:
  - a- Infection may be transmitted faeco-orally.
  - b- They are common causes of aseptic meningitis.
  - c- They replicate in the GIT.
  - d- They are important causes of diarrhoea.
  - e- They include polioviruses and coxsackieviruses.
  
- 2- Sabin poliovaccine can be considered superior to Salk vaccine in all of the following aspects **EXCEPT**:
  - a- Ease of administration
  - b- Safety
  - c- Ability of virus to pass through stools and be transmitted to non-immunized children
  - d- Ability of conferring local intestinal immunity
  - e- Ability to prevent all stages of the infection
  
- 3- Repeated infections of common cold could be explained by all of the following **EXCEPT**:
  - a- Multiple antigenic types
  - b- Nature of infection is superficial
  - c- Serum antibodies play no significant role
  - d- Rhinoviruses are slow viruses
  - e- Immunity is mainly superficial by IgA

## REOVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- Describe the structure of rotavirus
  - Summarize the laboratory diagnosis of infantile gastroenteritis caused by rotavirus
  - Summarize the prevention of rotavirus infection
- 
- The name “Reoviruses” stands for respiratory enteric orphan viruses.
  - They are non-enveloped viruses with a segmented dsRNA genome.
  - Rotavirus is the most important human pathogen in the reoviridae family.

## Rotavirus

The name “rotavirus” is derived from the characteristic wheel-like appearance of the virus by electron microscope (rota = wheel) (Fig. 39).

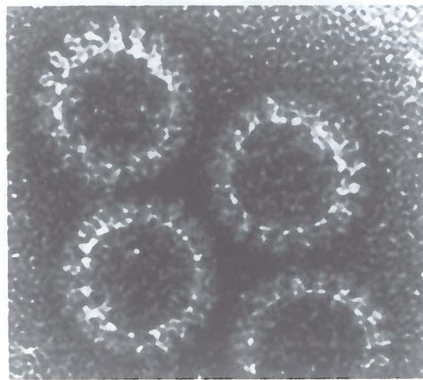


Fig. (39): Structure of Rotavirus

### Disease

- Rotavirus is the most important cause of **gastroenteritis in infants and young children**.
- The high incidence of rotavirus infection is attributed to:
  - Low infectivity dose (10 ingested viral particles).
  - Shedding of large number of virus particles in stools.
  - Relative stability of the virus allowing it to survive for extended periods on surfaces.

- The virus is transmitted **faeco-orally** following ingestion of contaminated food or water, by person-to-person contact, or by contact with contaminated surfaces.
- The incubation period is 1- 4 days.
- The disease manifests as watery non-bloody diarrhoea which may be fatal if untreated.

### Laboratory diagnosis

1. Detection of viral antigen in stools by ELISA.
2. Demonstration of rising titres of antibodies in patient serum by ELISA.
3. Examination of stools by electron microscope to detect the wheel-like viral particles. It is not routinely done.

### Treatment

Restoration of fluid and electrolyte balance.

### Prophylaxis

- Hygienic measures to control faeco-oral infections.
- Encouraging breast feeding which provides the newborn with protective IgA.
- Rotavirus vaccines:
  - Two oral vaccines are available:
    1. Live attenuated vaccine which contains the single most common rotavirus serotype.
    2. Live reassortant vaccine which contains 5 rotavirus strains. The gene for the outer surface protein of human rotavirus is inserted into a bovine strain of rotavirus (non-pathogenic to humans).
  - They are given during the first 6 months of life.
  - The outer surface protein elicits a protective local immune response in the GIT through IgA production.

### MCQs:

- 1- Which of the following is the most common cause of infantile gastroenteritis?
  - a- Rotavirus
  - b- Adenovirus
  - c- Norwalk virus
  - d- Poliovirus
  - e- Hepatitis A virus
- 2- True or false:
  - a- Rotavirus is a ds DNA virus.
  - b- The name "rotavirus" is derived from the characteristic rotatory movement of the virus.
  - c- Rotavirus has double stranded RNA genome.
  - d- The infective dose of rotavirus is very low.
  - e- Rotavirus is an enveloped virus.



## CALICIVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- Describe caliciviruses
  - State the pathogenesis and clinical features of gastroenteritis caused by Norovirus
  - Summarize the laboratory diagnosis and prevention of Norovirus infection
- 
- Caliciviruses are non-enveloped, ssRNA viruses.
  - The family includes two human pathogens:
    - Norovirus (previously known as Norwalk virus).
    - Hepatitis E virus (described previously).

### Norovirus (Norwalk virus)

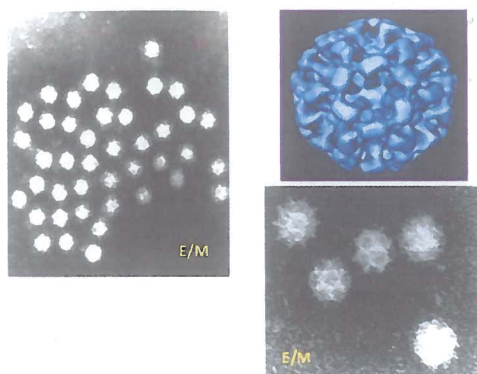


Fig. (40): Human Caliciviruses (Norwalk viruses)

- Norovirus is the most important cause of **epidemic viral gastroenteritis in school children and adults**.
- The virus was first discovered during an epidemic of gastroenteritis that occurred in Norwalk, Ohio, in 1968.
- Outbreaks typically occur in group settings such as camps, schools and nursing homes.
- Norovirus is similar to rotavirus as regards the mode of transmission, the incubation period, clinical manifestations and the high infectivity.
- The disease is self-limited; recovery occurs within 24-48 hours

## Diagnosis

- Diagnosis is often a clinical one.
- Laboratory diagnosis is important during outbreaks. It may be done serologically or by PCR.

## Prevention

- No specific vaccine is available for Norovirus gastroenteritis.
- Hand washing and careful monitoring of water purification are the most important measures in the control of infection.

## MCQs:

- 1- Regarding Norovirus, all the following statements are true EXCEPT:
- a- It is a non-enveloped virus.
  - b- It has ssRNA genome.
  - c- It is the most important cause of infantile gastroenteritis.
  - d- It is highly infective.
  - e- No specific vaccine is available.

## HERPESVIRUSES

### **ILOs:**

**By the end of this chapter, the student should be able to:**

- Identify the different members of herpesviruses
- Compare and contrast the types of herpes simplex virus (HSV)
- Explain the pathogenesis of HSV infection
- Summarize the clinical picture of HSV infection
- List the laboratory diagnosis of HSV infection
- Identify the treatment of HSV infection
- Summarize the prevention of HSV infection
- Identify the pathogenesis and clinical findings of chickenpox
- Identify pathogenesis and clinical findings of shingles
- List the treatment of varicella and zoster
- Summarize the prevention and control of varicella and zoster
- List Epstein-Barr virus (EBV)-associated diseases
- Summarize the pathogenesis and clinical features of glandular fever
- Identify the laboratory diagnosis of glandular fever
- State the modes of transmission of cytomegalovirus (CMV)
- Identify the clinical features of CMV infection
- Summarize the laboratory diagnosis of CMV infection
- Identify the treatment and prevention of CMV infection
- Describe diseases caused by Human Herpes Virus-6
- Describe diseases caused by Human Herpes Virus-7
- Describe diseases caused by Human Herpes Virus-8

- Herpesviruses are large enveloped dsDNA viruses, with icosahedral symmetry (Fig. 41)
- They replicate in the nucleus of infected cells; hence:
  - they form intranuclear inclusion bodies, and
  - derive their envelope from the nuclear membrane during budding.
- Herpesviruses have the capacity to establish life-long **latent** infections.



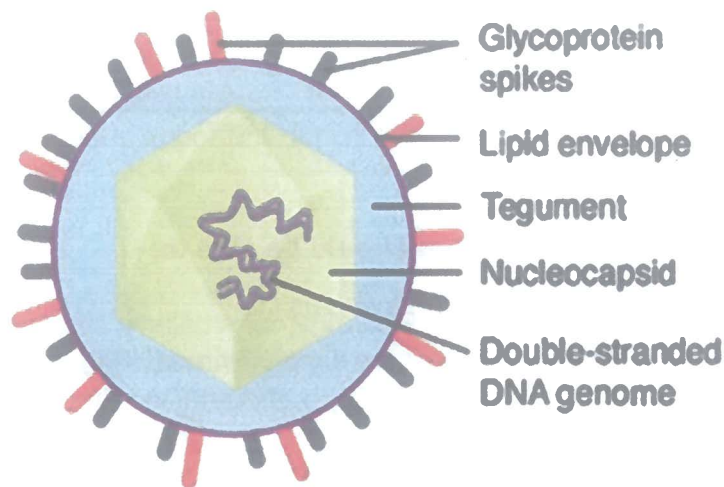


Fig. (41): Herpes virus

### Herpesviruses of medical importance

1. Herpes simplex virus-1 and 2 (HSV-1 and HSV-2).
2. Varicella-Zoster virus (VZV).
3. Epstein-Barr virus (EBV).
4. Cytomegalovirus (CMV).
5. Human herpesvirus-6 (HHV-6).
6. Human herpesvirus-7 (HHV-7).
7. Human herpesvirus-8 (HHV-8).

### Herpes Simplex Virus (HSV)

There are two antigenically distinct types of HSV: HSV-1 and HSV-2. In general, lesions caused by HSV-1 are above the waist, whereas those caused by HSV-2 are below the waist.

### Pathogenesis

- **Mode of transmission**
  - HSV-1 is transmitted primarily by **saliva** (e.g. by kissing)
  - HSV-2 is transmitted by **sexual** contact and **vertically** during birth.
- **Primary infection:**  
It occurs as a result of viral replication in the mucosal epithelial cells at the initial site of infection with the formation of vesicles.
- **Latency (Fig. 42):**  
The virus particles enter sensory nerve endings in the lesion and are transported to the regional ganglia (trigeminal ganglion in case of HSV-1 and sacral ganglia in case of HSV-2) to establish a life-long latent infection.
- **Reactivation:**
  - Reactivation of the latent virus may occur by a variety of inducers (e.g., fever, sunlight, hormonal changes, trauma, and stress).

- The virus migrates down the sensory nerves causing lesions at any site innervated by the affected neurons.
- The presence of circulating antibody does not prevent the recurrence but reduces the severity and limits the spread of virus to surrounding tissue.
- Reactivation of the latent virus may be restricted to asymptomatic shedding of the virus only.

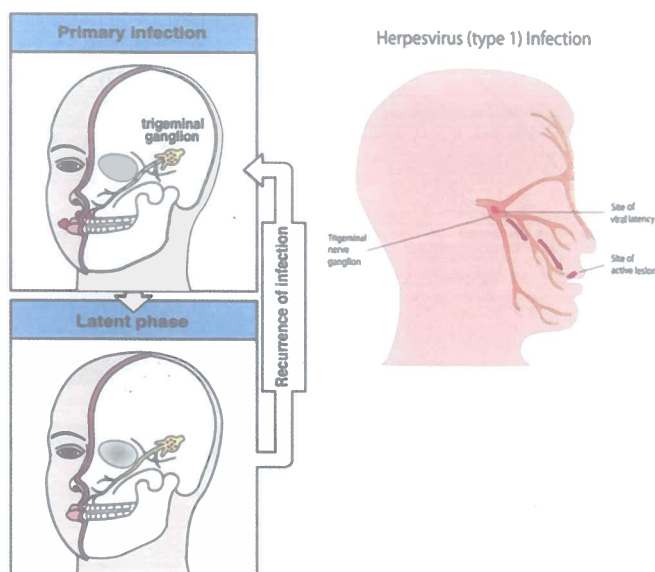


Fig. (42): Viral latency of HSV

## Clinical presentation

### I- HSV-1 infections (Fig. 43)

1. **Acute Gingivostomatitis:** It is the commonest classic presentation. It occurs primarily in children. It is characterized by fever and vesicular lesions in the mouth which heal without scar formation.
2. **Herpes labialis:** It is the milder recurrent form of the primary gingivostomatitis. It is also referred to as "fever blisters" or "cold sores".
3. **Keratoconjunctivitis:** It may lead to corneal ulcers, scarring and blindness.
4. **Encephalitis:** It is the most serious infection.
5. **Disseminated infections:** e.g. pneumonia as in AIDS patients.
6. **Herpetic whitlow:** It is a pustular lesion of the finger or hand (often affecting dentists).



Herpetic whitlow

Fig. (43): HSV-1 diseases

## II- HSV-2 infections (Fig. 44)

1. **Herpes genitalis:** It is the classic presentation. It manifests as extensive bilateral painful vesicular lesions in the genital area, accompanied by fever, dysuria and inguinal lymphadenopathy.
2. **Neonatal herpes:** It is acquired during birth, and varies from asymptomatic infection to severe disease (e.g. disseminated lesions or encephalitis).
3. **Aseptic meningitis:** It is usually a mild disease.



Fig. (44): HSV-2 diseases

### Laboratory diagnosis

A presumptive diagnosis is made on the basis of clinical findings. Laboratory diagnosis is important, however, to prevent neonatal infection, encephalitis and keratoconjunctivitis in which early initiation of therapy is essential, yet characteristic lesions are not present.

Specimens include vesicle fluids, saliva, genital or conjunctival swabs, CSF....etc.

Diagnosis may be done by:

1. Isolation of the virus on tissue culture and detection of intra-nuclear inclusions.
2. Detection of viral DNA by PCR.
3. Detection of viral antigen by direct immunofluorescence which can also distinguish HSV-1 from HSV-2.
4. Serological tests can be used in the diagnosis of primary infection. Recurrence rarely causes a rise in antibody titre.

### Treatment

- a- **Acyclovir:** is a nucleotide analogue that inhibits viral DNA synthesis. It shortens the duration of the lesions and reduces shedding of the virus, but does not cure the latent state.
- b- Other drugs, e.g., famciclovir and penciclovir are alternatives if acyclovir-resistance develops.

### Prevention

- Avoiding contact with herpetic lesions.
- Immunocompromised patients, e.g., transplant recipients, are given acyclovir to prevent viral reactivation.
- Caesarean section is recommended for mothers with genital HSV infection to avoid neonatal infection.



## Varicella-Zoster Virus (VZV)

- VZV has only one antigenic type.
- Primary infection with VZV causes varicella (chickenpox), whereas reactivation of the latent virus causes zoster (shingles).

### Pathogenesis (Fig. 45)

- The virus is transmitted by respiratory droplets.
- VZV infects mucosa of the upper respiratory tract. It replicates in the regional lymph nodes and then spreads via the blood to the skin where the typical rash of chickenpox appears.
- The virus enters the cutaneous sensory neurons and migrates to the dorsal root ganglion where it enters a latent state. Reactivation of the virus may occur resulting in vesicular lesion and the severe nerve pain of zoster.
- Immunity following varicella is life-long; a person gets varicella only once. However, zoster can occur despite this immunity to varicella.

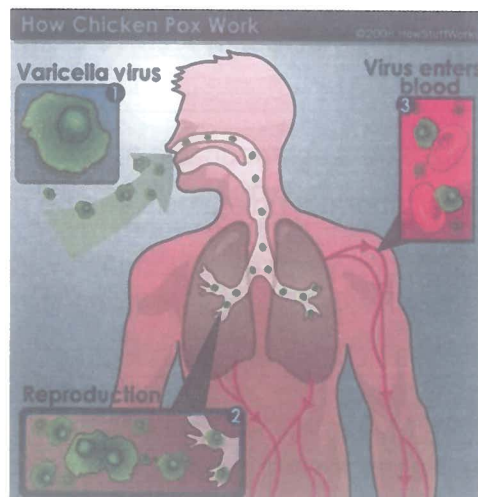


Fig. (45): Pathogenesis of Chickenpox

### Clinical manifestations

#### 1- Varicella (chickenpox):

- The incubation period is 2-3 weeks.
- Prodromal symptoms of fever and malaise occur, followed by the appearance of typical papulovesicular rash. The rash appears first on the trunk and then spreads to the head and extremities (Fig. 46).
- The vesicular lesions (containing the virus) dry to form crusts which heal without scar formation.
- Varicella occurs mainly in children and is usually mild. It is more severe and more likely to cause complications (e.g., pneumonia or encephalitis) if it occurs in adults.
- Pregnant women infected in the first half of pregnancy may pass infection to the foetus resulting in **congenital varicella syndrome** with high mortality rate.

## 2- Zoster (shingles):

- It results from reactivation of latent varicella infection in the neurons that may follow stress, immunosuppression or local trauma.
- The disease manifests as unilateral painful vesicular eruption restricted to one dermatome, usually thoracic or lumbar (Fig. 47) .
- The disease usually occurs in older people.



Fig. (46): Chickenpox



Fig. (47): Zoster

## Diagnosis

Diagnosis is mainly clinical, however, laboratory diagnosis can be done on the same lines used for HSV.

## Treatment

- Treatment is often not considered, as chickenpox is thought of as a mild infection.
- However, antiviral therapy is indicated in severe or complicated cases, in immunocompromised patients, and in people with zoster.
- **Acyclovir** is the drug of choice. Famciclovir and penciclovir are alternatives.

## Prevention

### 1. Vaccination:

There are 2 live attenuated vaccines:

- **Varivax:** It is used to prevent varicella. It is given in 2 subcutaneous doses to children between 1-12 years of age.
- **Zostavax** (high potency version of varivax): It is recommended to old people who have had varicella to reduce the risk of developing zoster. It is given in one subcutaneous dose.

### 2. VZ immune globulin (VZIG):

It is recommended for post-exposure prophylaxis of immunocompromised individuals or neonates born to infected mothers.

## Epstein-Barr virus (EBV)

Although infection with EBV is widespread, most infections are asymptomatic.

### Diseases

1. Infectious mononucleosis (glandular fever).
2. EBV-associated diseases:
  - Burkitt's lymphoma.
  - Nasopharyngeal carcinoma.
  - Oral hairy leukoplakia (seen in AIDS patients)
  - Hodgkin's disease.
  - T-cell lymphoma.

## Infectious Mononucleosis (Glandular Fever)

### Mode of transmission

EBV is transmitted by intimate contact with **saliva** (kissing disease).

### Pathogenesis (Fig. 48)

- After an initial replication in the oro-pharyngeal epithelium, the virus invades the blood stream where it infects B lymphocytes.
- Cytotoxic T lymphocytes react against the infected B cells, resulting in appearance of atypical T lymphocytes in the peripheral blood.
- The immunological response is associated with the production of cytokines (including B cell growth factors) resulting in multiplication and immortalization of B cells rather than cell death.
- Thus, infection induces a polyclonal B cell activation and production of heterophil antibodies and a variety of autoantibodies.
- EBV remains latent in the infected B lymphocytes.

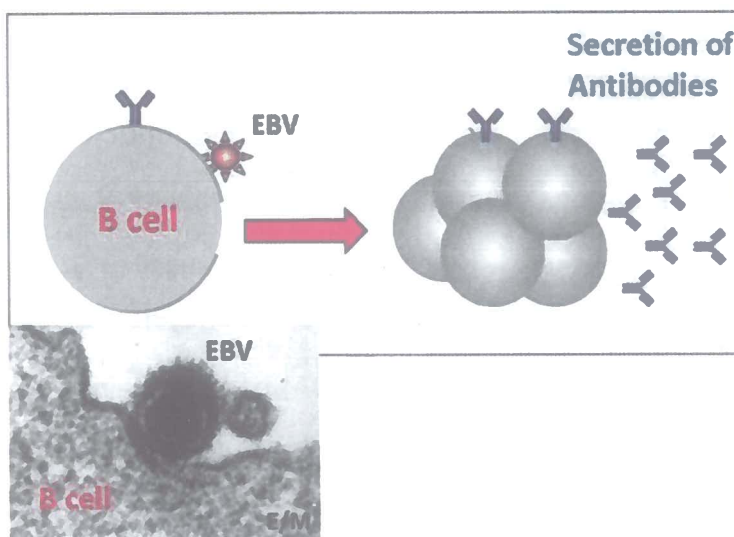


Fig. (48): B cell infection by EBV & Antibody secretion



### Clinical features

- After an incubation period of 4-7 weeks, the patient complains of fever, sore throat, skin rash, and cervical lymphadenopathy which becomes generalized with or without hepatosplenomegaly (Fig. 49).
- Spontaneous recovery usually occurs in 2-3 weeks.

Mononucleosis  
causes:

- Fever
- Fatigue
- Sore throat
- Swollen lymph glands

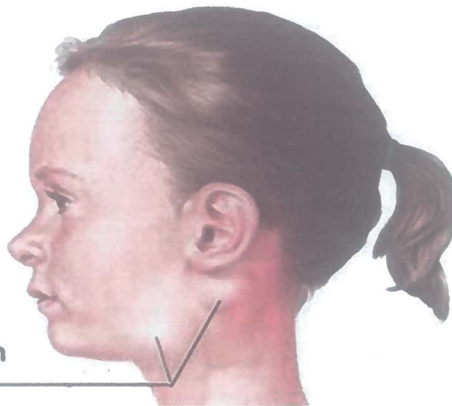


Fig. (49): Clinical manifestations of Infectious mononucleosis ("Kissing Disease")

### Laboratory diagnosis

1. Blood picture: high total leucocytic count (up to 25,000/cmm) with the presence of atypical lymphocytes (Fig. 50).

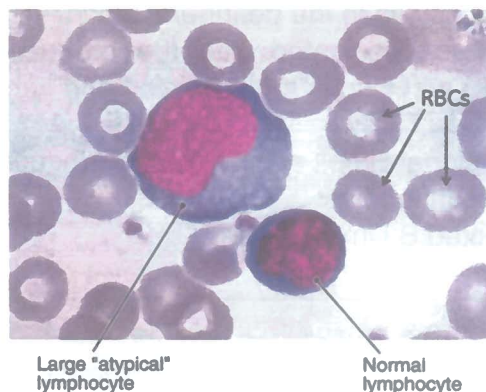


Fig. (50): Blood picture from a case of infectious mononucleosis

2. Detection of **heterophil antibodies** which agglutinate horse or sheep red cells (Paul Bunnell test or monospot test).
3. Detection of **EBV specific antibodies**:
  - Demonstration of IgM or rising titre of IgG to viral capsid antigen (VCA) indicates acute infection.
  - Demonstration of IgG to viral capsid antigen (VCA) or to Epstein Barr nuclear antigen (EBNA) indicates past infection.
4. Detection of EBV nucleic acid using PCR.

### Treatment

No effective treatment is available for EBV diseases

## Cytomegalovirus (CMV)

- The name "cytomegalovirus" refers to the typically swollen CMV-infected cells.
- Primary infection occurs during childhood and the virus persists in the host for life (latent infection). Reactivation is common.
- The virus is widespread and more than 80% of adults have antibody against the virus.

### Mode of transmission

In infected individuals, the virus is shed in all body fluids; therefore, transmission may occur by the following routes: contact with saliva (the most common source), transplacental transfer, breast feeding, sexual contact, blood transfusion and organ transplantation.

### Pathogenesis

- Initial replication of the virus occurs in the epithelial cells of the respiratory and gastrointestinal tracts. This is followed by viraemia and systemic spread within infected lymphocytes and monocytes to all organs of the body particularly salivary glands and kidney.
- Latency is probably established in mononuclear cells with repeated episodes of asymptomatic virus shedding.

### Clinical features:

1. Most infections are asymptomatic.
2. Infection in **healthy** individuals may result in a mononucleosis-like syndrome clinically identical to that caused by EBV; however, heterophil antibodies are absent.
3. Infection in **immunocompromised** patients (particularly transplant recipients) may manifest as pneumonia, encephalitis, hepatitis, retinitis ... etc.
4. Primary infection **during pregnancy** results in "cytomegalic inclusion disease" in about 10% of infected fetuses. The most common features of this syndrome are mental retardation, microcephaly, hepatosplenomegaly, thrombocytopenia and blindness. Stillbirth or abortion may also occur.  
Reactivation of infection during pregnancy may also lead to foetal infection, but rarely to congenital abnormalities due to the presence of maternal antibodies.

### Laboratory diagnosis

This may be done by detection of:

1. CMV IgM or rising titre of IgG by ELISA.
2. Viral DNA by PCR in tissues or body fluids (e.g., urine).
3. Intranuclear inclusions in tissue biopsy by histologic staining.

### Treatment

- Ganciclovir has been used successfully in treatment of CMV infections in immunosuppressed patients.
- Unlike HSV and VZV, CMV is largely resistant to acyclovir.

### Prevention and control

- Screening blood and organ donors and exclusion of seropositive ones.
- Anti-CMV immunoglobulin with ganciclovir is given prophylactically to patients following organ transplantation.

### Human Herpes Virus-6 (HHV-6)

**HHV-6** is the causative agent of a common disease of infancy called **roseola infantum** (exanthem subitum or sixth disease) which is characterized by high fever and skin rash. Transmission is via saliva.

### Human Herpes Virus-7 (HHV-7)

**HHV-7** is commonly present in the saliva of healthy individuals and infection of the salivary glands is frequently asymptomatic.

### Human Herpes Virus-8 (HHV-8)

**HHV-8** was identified in 1994 from tissue of **Kaposi's sarcoma** in patients with AIDS. It is transmitted sexually, by saliva, vertically and by organ transplantation.

**Table (9):** Properties of common herpesvirus infections

<b>Virus</b>	<b>Site of 1<sup>st</sup> infections</b>	<b>Common primary infections</b>	<b>Site of latency</b>	<b>Recurrent infections</b>
HSV-1	Mucosa	- Gingivostomatitis - Keratoconjunctivitis	Trigeminal ganglia	Herpes labialis (cold sores)
HSV-2	Mucosa	- Herpes genitalis - Neonatal herpes	Sacral ganglia	Herpes genitalis
VZV	Mucosa	- Varicella (chickenpox)	Dorsal root ganglia	Herpes zoster (shingles)
EBV	Mucosa, B lymphocytes	- Infectious mononucleosis - Burkitt's lymphoma	B lymphocytes	Asymptomatic shedding of virus
CMV	Mucosa, mononuclear cells	- Mononucleosis-like syndrome - Cytomegalic inclusion disease	Mononuclear cells	Asymptomatic shedding of virus



**MCQs:**

- 1- **Herpes simplex virus-1 (HSV-1) diseases include all of the following EXCEPT:**
  - a- Keratoconjunctivitis
  - b- Acute gingivostomatitis
  - c- Herpetic whitlow
  - d- Disseminated infections in AIDS patients
  - e- Genital infections
- 2- **Chickenpox is a common disease of childhood. It is caused by which of the following viruses?**
  - a- Adenovirus
  - b- Cytomegalovirus
  - c- Papillomavirus
  - d- Rotavirus
  - e- Varicella virus
- 3- **Which one of the following viruses is the leading cause of congenital malformations?**
  - a- Cytomegalovirus
  - b- Mumps virus
  - c- Poliovirus
  - d- Respiratory syncytial virus
  - e- Rhinovirus
- 4- **Laboratory findings in infectious mononucleosis include:**
  - a- Decreased total leucocytic count
  - b- Presence of heterophil antibodies
  - c- Decreased number of monocytes
  - d- Detection of EBV antigens using DNA probes
  - e- Detection of EBV antibodies using DNA probes
- 5- **This virus may be detected by polymerase chain reaction (PCR) in a variety of cells of patients with nasopharyngeal carcinoma:**
  - a- Measles virus
  - b- Mumps virus
  - c- Rubella virus
  - d- Parvovirus
  - e- Epstein-Barr virus
- 6- **The virus associated with Kaposi's sarcoma in AIDS patients is:**
  - a- HSV-2
  - b- EBV
  - c- HHV-6
  - d- HHV-7
  - e- HHV-8

## POXVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- Describe the important features of poxviruses
- List members of poxviruses
- Explain causes of successful eradication of smallpox
- Summarize the pathogenesis and the clinical features of molluscum contagiosum virus

- Poxviruses are large enveloped ds DNA viruses
- They differ from other DNA viruses in:
  - being brick-shaped (other DNA viruses are icosahedral) (Fig. 51)
  - replicating in the cytoplasm as they contain their own DNA-dependent RNA polymerase (other DNA viruses replicate in the nucleus)
  - being the only viruses which can be seen by the light microscope because of their large size (250-300 nm).

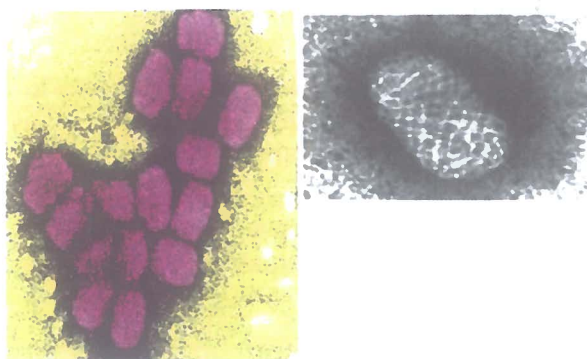


Fig. (51): Poxviruses

### Poxviruses include:

- Poxviruses are large enveloped ds DNA viruses
- They differ from other DNA viruses in:
  - being brick-shaped (other DNA viruses are icosahedral)
  - replicating in the cytoplasm as they contain their own DNA-dependent RNA polymerase (other DNA viruses replicate in the nucleus)
  - being the only viruses which can be seen by the light microscope because of their large size (250-300 nm).

## Smallpox Virus

Smallpox is the only disease that has been **eradicated** as declared by the WHO in 1980. However, there is concern regarding the use of smallpox virus as an agent of **bioterrorism**.

### Causes of successful eradication of smallpox:

1. The **global** use of a highly effective vaccine by the WHO. The vaccine contains vaccinia virus which is naturally attenuated for man.
2. Smallpox virus has a single, stable serotype.
3. There is no animal reservoir, and humans are the only host.
4. The disease is easily recognized clinically.
5. There is no carrier state or subclinical infection.
6. The **global** use of a highly effective vaccine by the WHO. The vaccine contains vaccinia virus which is naturally attenuated for man (fig. 52)
7. Smallpox virus has a single, stable serotype.
8. There is no animal reservoir, and humans are the only host.
9. The disease is easily recognized clinically.
10. There is no carrier state or subclinical infection.



Fig. (52): Smallpox

## Molluscum Contagiosum Virus

- Molluscum contagiosum virus is transmitted by close personal contact, including sexual contact.
- It causes wart-like benign tumours of the skin or mucous membranes (that differ from warts caused by papillomavirus). The lesions can be widespread in patients with reduced cell-mediated immunity.
- Infection is common in children.
- There is no specific antiviral treatment and no vaccine.

### MCQs:

- 1- All the following statements regarding eradication of smallpox by vaccine are true **EXCEPT**:
  - a- Smallpox has a single serotype.
  - b- There is no carrier state.
  - c- There is no subclinical infection.
  - d- Human is the only host.
  - e- The disease is difficult to be recognized clinically.



## ADENOVIRUSES

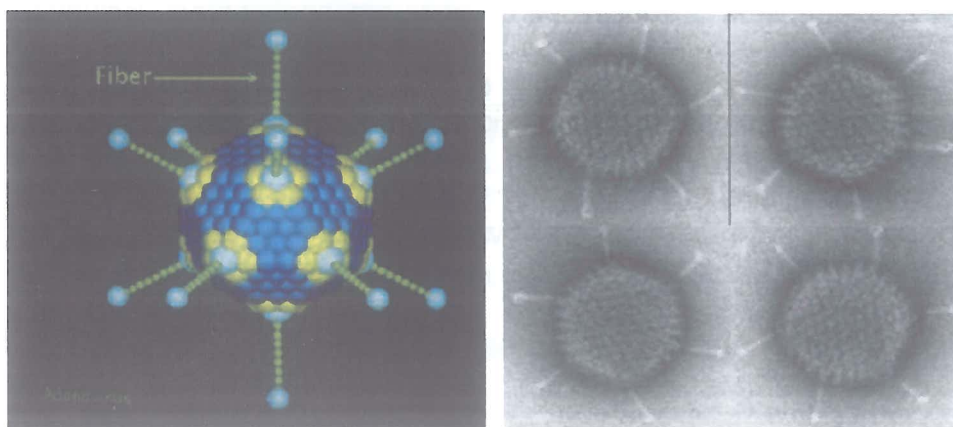
### ILOs:

**By the end of this chapter, the student should be able to:**

- State the characteristic features of adenoviruses
- Describe the morphology of adenoviruses
- Outline adenovirus infections in humans
- Recognize the laboratory diagnosis of adenoviruses infections
- Recognize the prevention of adenoviruses infections

Adenoviruses were isolated from adenoidal tissue of children, hence the name “adenovirus”.

- They are non-enveloped ds DNA viruses with an icosahedral symmetry.
- Adenoviruses are the only viruses with fibers protruding from the capsid. The fiber is the organ of attachment, a strong haemagglutinin and toxic to human cells (Fig. 53).
- Adenoviruses are widespread in nature as they are resistant to drying, detergents and mild chlorine treatment.
- Over 50 serotypes of human adenoviruses are known.
- Adenoviruses are used as cloning vectors for gene therapy.



**Fig. (53): Adenovirus**

### Pathogenesis

Adenoviruses are capable of causing:

- **Lytic infections:** e.g., in muco-epithelial cells.
- **Latent infection:** e.g., in lymphoid and adenoid tissues.
- **Cell transformation:** in experimental animals **not in humans**.

### Mode of transmission

- Respiratory droplets
- Direct contact
- Faeco-oral route

### Diseases

- The site of adenovirus infection is generally related to the mode of virus transmission. In addition, specific clinical syndromes are associated with certain adenovirus serotypes.
- Approximately 50% of the infections are asymptomatic, self-limiting, and induce long-lasting **type-specific** immunity.

#### A- Respiratory diseases:

1. Acute febrile pharyngitis.
2. Pharyngoconjunctival fever.
3. Acute respiratory disease occurring in epidemic form especially among military recruits. It is characterized by pharyngitis, fever, cough, and malaise.
4. Adenoviral pneumonia representing 10% of pneumonias in childhood.

#### B- Eye infections:

1. Swimming pool conjunctivitis (pink eye) (Fig. 54).
2. Follicular conjunctivitis resembling chlamydial conjunctivitis.
3. Epidemic keratoconjunctivitis is the most serious. The disease is highly infectious and may lead to corneal opacity.



Fig. (54): Adenovirus pink eye

#### C- Gastrointestinal diseases:

1. Infantile gastroenteritis.
2. Intussusception of infancy.

#### D- Other diseases: e.g.,

1. Acute haemorrhagic cystitis in children.
2. Meningitis.

### Laboratory diagnosis

- Isolation of the virus in cell culture.
- Serological diagnosis by ELISA.

### Treatment

Antiviral agents have generally been ineffective against adenovirus infections.

### Prevention

- Careful hand washing.
- Chlorination of swimming pools and drinking water.
- High hygiene standards in ophthalmology practice.

### MCQs:

- 1- Which one of the following viruses would be most likely to establish a latent infection?
  - a- Adenovirus
  - b- Measles virus
  - c- Influenza virus
  - d- Parvovirus
  - e- Coxsackievirus group B
- 2- Swimming pool conjunctivitis is caused by:
  - a- Adenovirus
  - b- Cytomegalovirus
  - c- Human papilloma virus
  - d- Epstein-Barr virus
  - e- Coronaviruses



## PARVOVIRUSES

### ILOs:

By the end of this chapter, the student should be able to:

- Identify the pathogenesis and clinical picture of parvovirus B19 infection
- Summarize the laboratory diagnosis of parvovirus B19 infection
- Summarize the treatment of parvovirus B19 infection

- Parvoviruses are the smallest of the DNA viruses (parvo = small).
- They are non-enveloped, ssDNA viruses with icosahedral symmetry.
- **Parvovirus B19** is the only member of the family that is a human pathogen.

### Mode of transmission

- Respiratory route.
- Transplacental transfer.
- Transfusion of blood or blood products.

### Pathogenesis

Parvovirus B19 infects 2 types of cells:

- Red blood cell precursors in the bone marrow, where the virus replicates inside the nucleus resulting in their lysis (Fig. 55).
- Vascular endothelial cells.

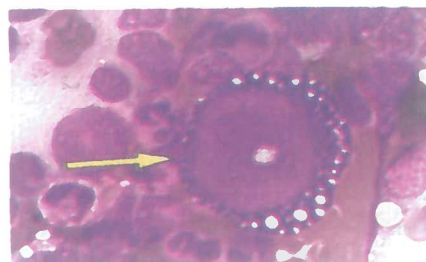


Fig. (55): Progenitor (nucleated) RBC with Parvovirus infection

### Diseases

Most of the infections are asymptomatic. Human diseases include:

1. Erythema infectiosum (slapped-cheeks syndrome, fifth disease\*). The rash associated with erythema infectiosum is due to infection of the endothelial cells as well as deposition of immune complexes (Fig. 56).
2. Aplastic anaemia: This occurs in children with chronic haemolytic anaemia (e.g., sickle cell anaemia). This aplastic crisis is the most serious complication.
3. Hydrops foetalis: The virus infects the foetal erythrocyte precursors causing severe anaemia. This may lead to congestive heart failure causing massive oedema of the foetus (Fig. 57).
4. Arthritis: It occurs in adults. It resembles rheumatoid arthritis and is caused by deposition of immune complexes.



Fig. (56): Erythema infectiosum

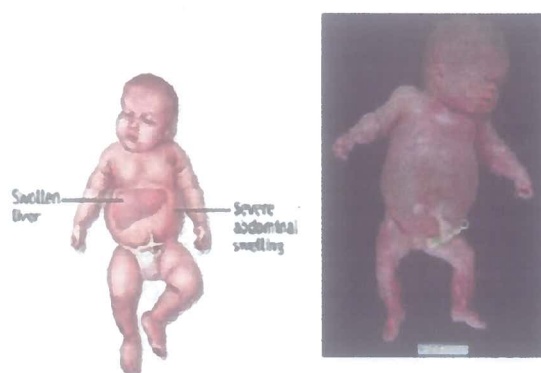


Fig. (57): Foetal hydrops

## Diagnosis

- Diagnosis is usually based on clinical presentation.
- Laboratory diagnosis may be done serologically or by PCR.

## Treatment

There is no specific antiviral drug.

## MCQs:

- 1- Which of the following is a virus that infects and lyses progenitor erythroid cells causing transient aplastic crisis in patients with haemolytic anaemia?
  - a- Encephalitis virus
  - b- Epstein-Barr virus
  - c- Parvovirus B19
  - d- Yellow fever virus
  - e- Cytomegalovirus
- 2- Which of the following is the causative viral agent of slapped cheek syndrome?
  - a- Ebola virus
  - b- Parvovirus B19
  - c- Hantaan virus
  - d- Cytomegalovirus
  - e- Adenoviruses
- 3- True or false:
  - a- Parvoviruses are large DNA viruses.
  - b- Parvoviruses are the only ssDNA viruses.
  - c- Parvovirus B19 infects the red blood cell precursors.
  - d- Parvovirus B19 can be transmitted transplacentally.
  - e- Parvovirus B19 is the causative agent of "fifth disease".

---

\* The 4 other childhood diseases associated with skin rash are measles, rubella, varicella and scarlet fever.

## HUMAN PAPILLOMAVIRUS

### ILOs:

By the end of this chapter, the student should be able to:

- Describe the characteristic features of human papillomaviruses
  - Identify the modes of transmission and pathogenesis of human papillomaviruses
  - Identify human papillomaviruses vaccine and state its nature, route and
- Human papillomavirus (HPV) is a non-enveloped dsDNA virus.
  - There are more than 100 HPV genotypes.
  - Important types include:
    - HPV 16 & 18 (the high risk types) associated with cervical cancer.
    - HPV 6 & 11 associated with ano-genital warts (condyloma acuminata).
    - HPV 1- 4 associated with cutaneous warts.

### Mode of transmission

Direct contact including sexual contact and materno-foetal during birth.

### Pathogenesis (Fig. 58)

- HPV infects the squamous epithelium.
- Hyperkeratosis leads to the formation of warts.
- The viral DNA may integrate in the host cell chromosome leading to over-expression of certain viral genes that encode certain viral proteins (E6 and E7). These proteins inactivate tumor suppressor proteins encoded by p53 and retinoblastoma (Rb) genes, and induce abnormal mitosis resulting in malignant transformation.
- E6 and E7 proteins of HPV 16 bind more strongly to p53 and Rb proteins than E6 and E7 proteins of other types not associated with carcinoma.

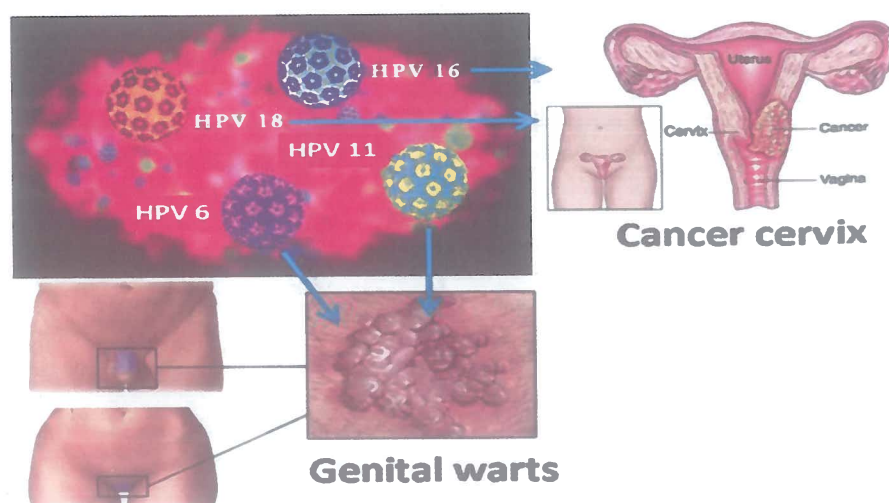


Fig. (58): HPV diseases



## Diagnosis

- HPV infections are usually diagnosed clinically.
- Lab diagnosis is done by examining cervical swabs or tissue biopsy by:
  - a. Cytology (Pap smear)
  - b. PCR which can be used for detection and typing of HPV.

## Prevention

Two recombinant vaccines containing the HPV capsid proteins are available:

- **Bivalent vaccine:** against high risk HPV types, 16 and 18. It protects against cervical carcinoma.
- **Tetravalent vaccine:** against HPV types 6, 11, 16 and 18. It protects against development of both cervical cancers and ano-genital warts.

They are recommended starting at age 11 years (before sexual activity). They are administered intramuscularly in 3 doses at 0, 1, 6 months.

## MCQs:

- 1- **Human papillomavirus vaccine:**
  - a- Is a quadrivalent vaccine that prevents infection by types 6, 11, 16 and 18
  - b- Is routinely given to newborn females during their first year of life
  - c- Is a living attenuated vaccine
  - d- Is given mainly to post-menopausal women
  - e- Protects against development of ovarian cancer
- 2- **True or false:**
  - a- HPV has a single stable genotype.
  - b- HPV is transmitted sexually and perinatally.
  - c- HPV 16 and 18 are high-risk types.

## TUMOUR VIRUSES AND ONCOGENESIS

### ILOs:

By the end of this chapter, the student should be able to:

- Recognize cellular transformation and oncogenesis
  - List evidences for the causal relationship between viruses and human cancer
  - Identify the mechanisms of cell transformation
  - List the tumour viruses and the type of malignancy linked to each of them
- Tumour viruses, also called oncoviruses, are those viruses which when introduced into an appropriate host can produce tumour.
  - It is well proved that viruses cause cancer in animals. However, proving causal relationship between viruses and human cancer is controversy and is usually based on evidences as:
    1. Isolation of the virus from tumour cells at some stages of development.
    2. Detection of viral nucleic acid or viral encoded protein in tumour cells.
    3. The ability of the virus to cause transformation of cultured cells or tumour in laboratory animals.
    4. Decrease in incidence of some tumours by prevention of the related virus.
  - Tumour viruses can cause cellular transformation which is a stable heritable change of cell characters.
  - Transformed cells contain either the whole or part of the viral genome usually integrated into the cell DNA (Fig. 59).
    1. DNA viruses: A portion of viral DNA becomes integrated in the host cell genome.
    2. RNA viruses (e.g., retroviruses): The reverse transcriptase makes a DNA copy of the viral RNA which is then integrated into the cell genome.

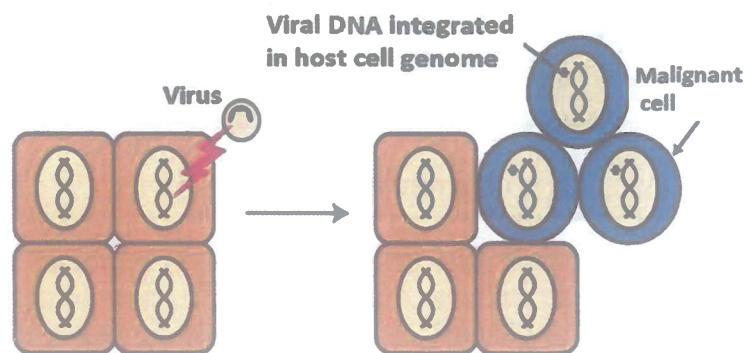


Fig. (59): Mechanism of cell transformation by oncogenic viruses

- The integrated viral genes then cause transformation through:
  1. Introduction of a new "transforming gene" into the cell (**viral oncogene**).
  2. Activation of a preexisting cellular gene (**proto-oncogene**).
  3. Inactivation of tumour suppressor genes (**anti-oncogenes**). The Rb gene and the p53 gene are altered in at least 50% of human tumours.
- Examples of tumour viruses and the associated tumours are shown in table (10).

**Table (10):** Tumour viruses and the associated tumours

	<b>The virus</b>	<b>The associated tumour</b>
<b>DNA Tumour Viruses</b>	Herpes simplex viruses 1 & 2	Cancer cervix
	Epstein Barr virus	Burkitt's lymphoma
	Human herpes virus-8	Kaposi's sarcoma
	Hepatitis B virus	Hepatocellular carcinoma
	Human papillomavirus	Cancer cervix
	Adenoviruses	Tumours in <b>rodents</b>
	Molluscum contagiosum virus	Benign skin warts
<b>RNA Tumour Viruses</b>	Hepatitis C virus	Hepatocellular carcinoma
	Human T-cell lymphotropic virus	T cell leukemia

**MCQs:**

- RNA tumour viruses include:**
  - a- Hepatitis B virus
  - b- Adenoviruses
  - c- Hepatitis C virus
  - d- Poxviruses
  - e- Human papillomavirus
- All the following are DNA tumour viruses EXCEPT:**
  - a- Adenoviruses
  - b- Human papillomavirus
  - c- Hepatitis B virus
  - d- Hepatitis A virus
  - e- Epstein Barr virus



## PRIONS

### ILOs:

By the end of this chapter, the student should be able to:

- Identify prions
  - State the characteristics of prions
  - Summarize the pathogenesis of prion diseases
  - List the clinical features of prion diseases
  - Compare between prions and conventional viruses
- Prions are unconventional infectious agents composed entirely of proteins. They are devoid of any nucleic acid.
  - They are encoded by cellular genes.
  - The normal prion proteins (known as  $\text{PrP}^{\text{C}}$ , or prion protein cellular) are expressed predominantly on the surface of nerve cells.
  - $\text{PrP}^{\text{C}}$  are in the normal alpha-helix configuration, and are non-pathogenic. But when their configuration changes to beta-sheet (known as  $\text{PrP}^{\text{Sc}}$ , or prion protein scrapie), they convert to the pathogenic form. Unlike the  $\text{PrP}^{\text{C}}$  which is protease sensitive,  $\text{PrP}^{\text{Sc}}$  is protease resistant (Fig. 60).

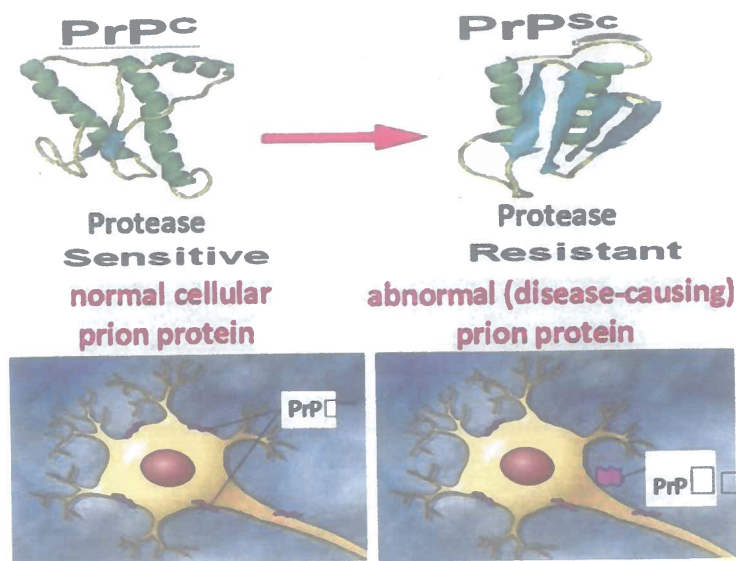


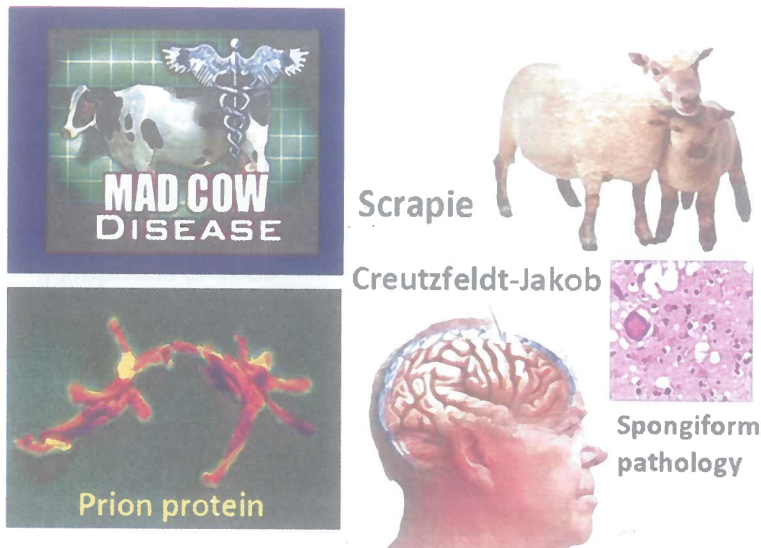
Fig. (60): Prions

- These abnormal forms ( $\text{PrP}^{\text{Sc}}$ ) aggregate into filaments which disrupt neuron function and cause cell death. The dead neurons cause vacuoles in the brain tissue giving it a sponge-like appearance. Therefore, prion diseases are called transmissible **spongiform encephalopathies**.

- Examples of prion diseases are shown in table 11 and figure 61.

**Table (11):** Prion diseases in man and animals

Disease	Primary Host
Creutzfeldt-Jacob disease (CJD)	Man
Kuru	Man
Scrapie	Sheep
Bovine spongiform encephalopathy (mad cow)	Cattle

**Fig. (61):** Prion diseases

### Characteristics of prion diseases

- Prion diseases have long incubation period, gradual onset and progressive fatal course.
- Prions are transmitted by ingestion of diseased brain and other CNS tissues or eye balls. Corneal transplant and use of brain electrodes are other methods of transmission.
- Most cases occur as sporadic disorders. About 10-15% occur as inherited disorders.
- The clinical features are progressive cerebellar syndrome with dementia in the final stages of illness.
- There is no evidence of host inflammatory reactions or immune response (due to lack of antigenicity).
- There is no antimicrobial therapy for these diseases.
- Prevention of transmission of prion infections by the usual infection control procedures is ineffective due to the extreme resistance of prions to heat, disinfectants and irradiation. However, prions are susceptible to autoclaving at 134°C for 1 hour, or exposure to high concentration of sodium hypochlorite, sodium hydroxide (1N solution) or phenol.

A comparison between prions and conventional viruses is shown in table 12.

**Table (12):** Comparison of prions and conventional viruses

Feature	Prions	Conventional viruses
Nucleic acid	Absent	Present
Proteins encoded by	Cellular genes	Viral genes
Inactivation by UV light or heat	No	Yes
Electron microscope appearance	Filamentous rods	Special symmetry
Cultivation	Non-cultivable	On cell lines
Induction of immune response	No	Yes
Induction of inflammatory response	No	Yes

### **MCQs:**

#### **1- Prions:**

- a- Contain DNA as genetic element
- b- May cause infections involving the upper respiratory tract
- c- Are easily inactivated by low concentrations of disinfectants
- d- Cause degenerative diseases of the nervous system
- e- Cause diseases characterized by short incubation period

#### **2- Mad cow disease is caused by:**

- a- A prion
- b- A virus
- c- Rickettsiae
- d- An autoimmune reaction
- e- A bacterium with a defective cell wall



# Viral infections

## Encephalitis/ meningitis

- JC virus
- Measles
- LCM virus
- Arbovirus
- Rabies

## Common cold

- Rhinoviruses
- Parainfluenza virus
- Respiratory syncytial virus

## Eye infections

- Herpes simplex virus
- Adenovirus
- Cytomegalovirus

## Pharyngitis

- Adenovirus
- Epstein-Barr virus
- Cytomegalovirus

## Gingivostomatitis

- Herpes simplex type 1

## Parotitis

- Mumps virus

## Pneumonia

- Influenza virus, Types A and B
- Parainfluenza virus
- Respiratory syncytial virus
- Adenovirus
- SARS coronavirus

## Cardiovascular

- Coxsackie B virus

## Hepatitis

- Hepatitis virus types A, B, C, D, E

## Myelitis

- Poliovirus
- HTLV-I

## Skin infections

- Varicella zoster virus
- Human herpesvirus 6
- Smallpox
- Molluscum contagiosum
- Human papillomavirus
- Parvovirus B19
- Rubella
- Measles
- Coxsackie A virus

## Gastroenteritis

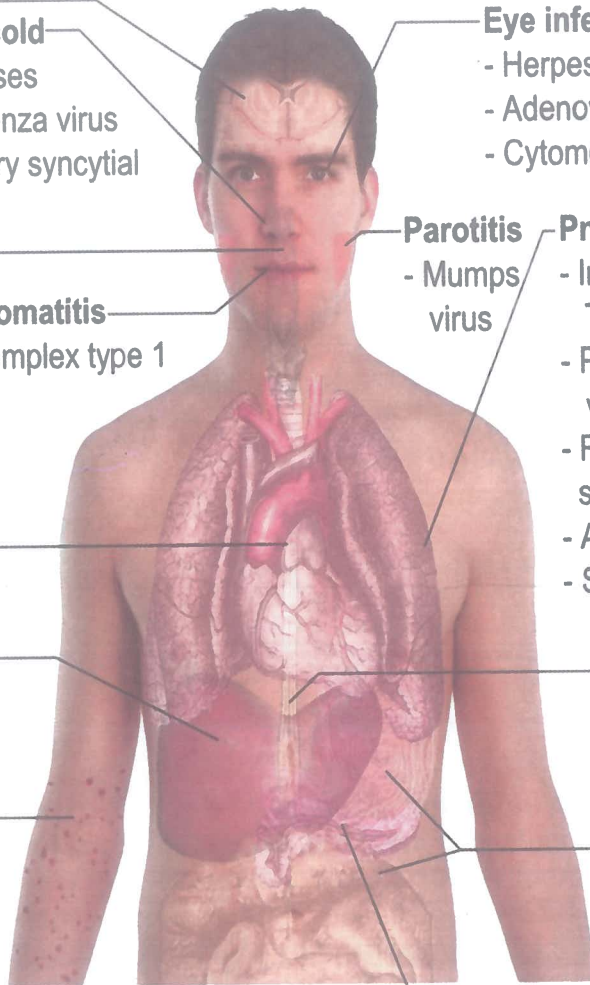
- Adenovirus
- Rotavirus
- Norovirus
- Astrovirus
- Coronavirus

## Sexually transmitted diseases

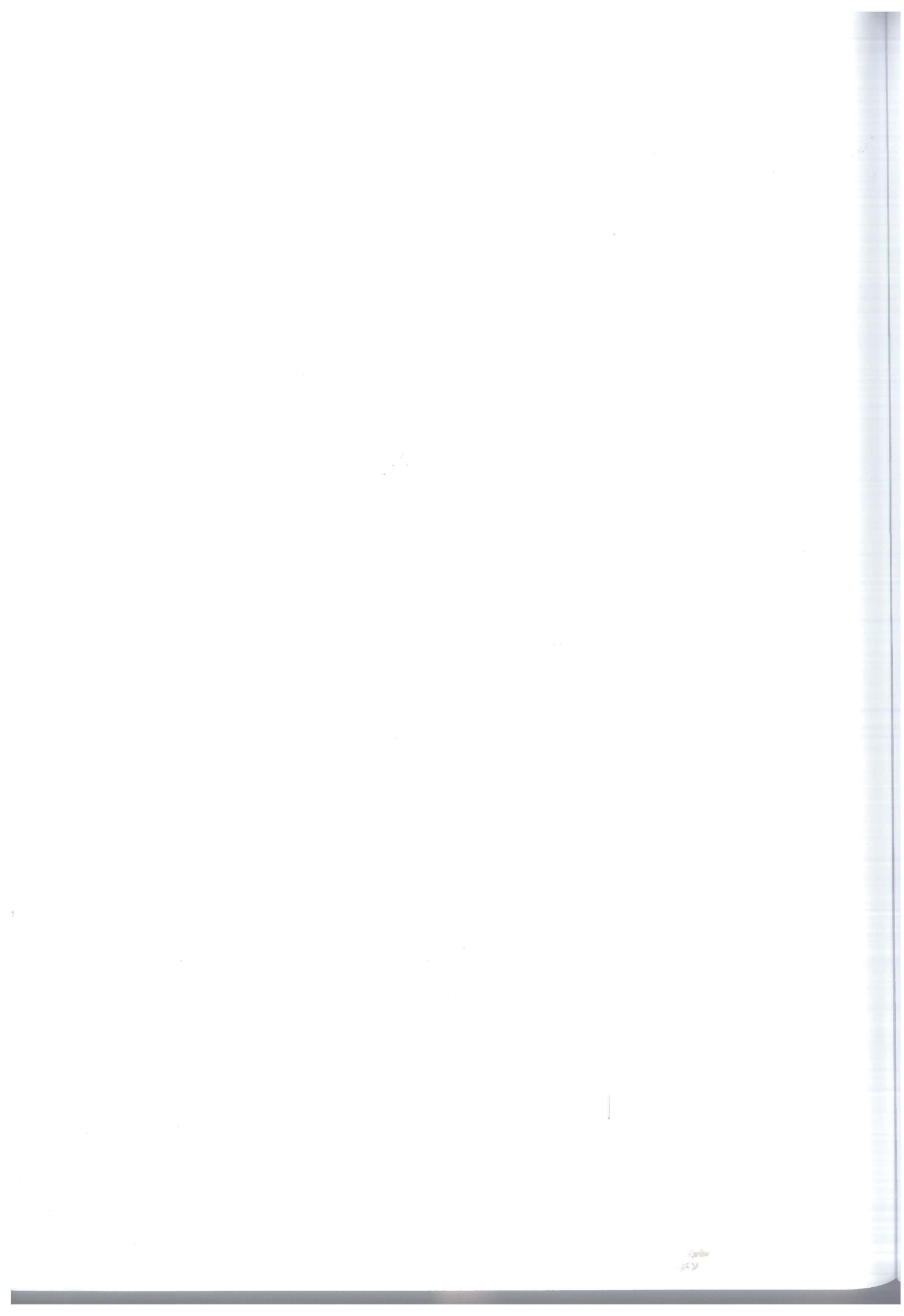
- Herpes simplex type 2
- Human papillomavirus
- HIV

## Pancreatitis

- Coxsackie B virus



# **SYSTEMATIC MYCOLOGY**





## SYSTEMATIC MYCOLOGY

### **ILOs:**

**By the end of this chapter the student should be able to:**

- Enumerate clinically important dermatophytes
- List predisposing factors, clinical forms and diagnosis of candidiasis
- List clinical forms of aspergillosis
- Outline pathogenesis and major laboratory diagnostic criteria of cryptococcosis
- Outline pathogenesis and differences between eumycotic and actinomycotic mycetoma

### ***Pityriasis versicolor***

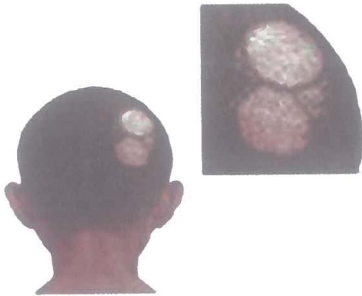
- It is caused by *Malassezia furfur*.
- It is a common fungus infection of the horny layer of the skin.
- It affects the upper part of the trunk and sides of the neck.
- It appears as scaly macules, either hyper- or hypopigmented.
- 

### **Dermatophytes**

- Three genera of dermatophytes infect man. These are: *Microsporum*, *Trichophyton* and *Epidermophyton*.
- They affect the keratinized tissue (hair, nail and skin).
- The sources of infection may be man, animals as dogs and cats, and soil.
- Infection is transmitted by direct or indirect contact.
- The disease is characterized by being superficial, extends radially and heals at the centre to form a circular lesion called **ringworm** which is usually referred to as **tinea** (Fig. 62).

**Dermatophytes****Fig. (62): Ringworm (healing center)**

- According to the site, there are several types of ringworm infections:
  1. Tinea capitis (ringworm of the scalp) (Fig. 63)
  2. Tinea pedis (athlete's foot) (Fig. 64)
  3. Tinea unguinum (ringworm of nails) (Fig. 65)

**Fig. (63): Tinea capitis****Fig. (64): Tinea pedis****Fig. (65): Tinea unguinum**

4. Tinea circinata (ringworm of non-hairy skin) (Fig. 66)
5. Tinea cruris (ringworm of the skin of the groin) (Fig. 67)
6. Tinea barbae (ringworm of the skin of beard) (Fig. 68)

**Fig. (66): Tinea circinata****Fig. (67): Tinea cruris****Fig. (68): Tinea barbae**

## Candidiasis

Candidiasis (moniliasis) is most frequently caused by *Candida albicans* and rarely due to infection by other species, e.g. *C. stellatoidea*, *C. krusei*...etc. *Candida albicans* is present as normal flora in the oral cavity, vagina and intestine.

### Predisposing factors to candidiasis include:

1. Diabetes.
2. Broad-spectrum antibiotic treatment (superinfection).
3. Steroid therapy.
4. Immunosuppression.
5. Prolonged exposure to water, pregnancy and old age.

### Clinical features of candidiasis

#### A. Cutaneous: Affecting skin and mucous membranes:

- Oral thrush (Fig. 69)
- Vulvovaginitis
- Paronychia (nail infection) (Fig. 70)
- Interdigital
- Intertrigo (between skin folds), e.g. diaper rash
- Chronic mucocutaneous candidiasis commonly associated with T-cell deficiency (Fig. 71)



Fig (69): Oral thrush    Fig. (70): Paronychia    Fig. (71): Mucocutaneous Candidiasis

**B. Systemic:** Affecting the lung or kidney. It usually complicates an underlying disease as TB, malignancy or immunosuppression.

### Laboratory diagnosis

1. **Specimens** from skin, mouth, vagina, sputum...etc.
2. **Microscopic examination** of a Gram-stained smear for the presence of Gram-positive, oval, budding yeast cells and pseudohyphae (Fig. 72).



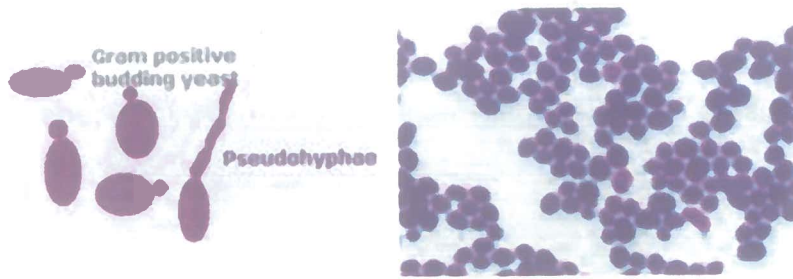


Fig. (72): Candida (Gram stain)

### 3. Cultivation:

- *C. albicans* can grow on most culture media at 37°C. The selective medium is Sabouraud's dextrose agar (SDA) containing chloramphenicol.
- After 1-2 days, yeast colonies appear creamy white, pasty with yeasty odour (Fig. 73).



Fig. (73): Candida on Sabouraud's dextrose agar (SDA)

- *C. albicans* is differentiated from other *Candida* species by:
  - Formation of germ tubes (true hyphae with no constrictions) in serum at 37°C within 1-2 hours (Fig. 74).
  - Formation of chlamydospores (thick-walled resting spores) on corn meal agar (Fig. 75).

Fig. (74): *Candida albicans* (germ tubes in serum)Fig. (75): *C. albicans* (chlamydospores) on corn meal agar

## Aspergillosis

- It is caused by *Aspergillus fumigatus*, rarely by *A. niger* and *A. flavus* (Fig. 76).

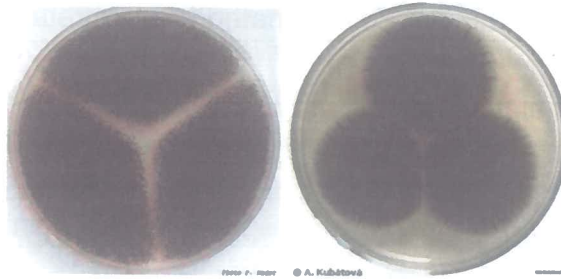


Fig. (76): *Aspergillus*

- The spores are found in air and are continuously inhaled.
- Aspergillosis may present as:
  - Allergy, e.g. bronchial asthma (type I hypersensitivity) (Fig. 77).
  - Non-invasive infections, e.g. aspergilloma (fungus ball) in a preexisting lung cavity (Fig. 78).

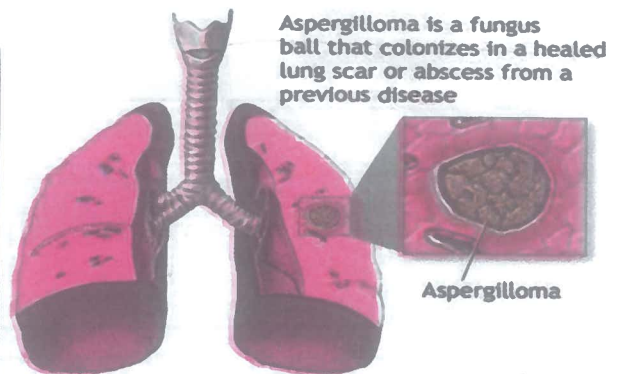


Fig. (77): Bronchial asthma (Aspergillosis)

Fig. (78): Non-invasive Aspergillosis

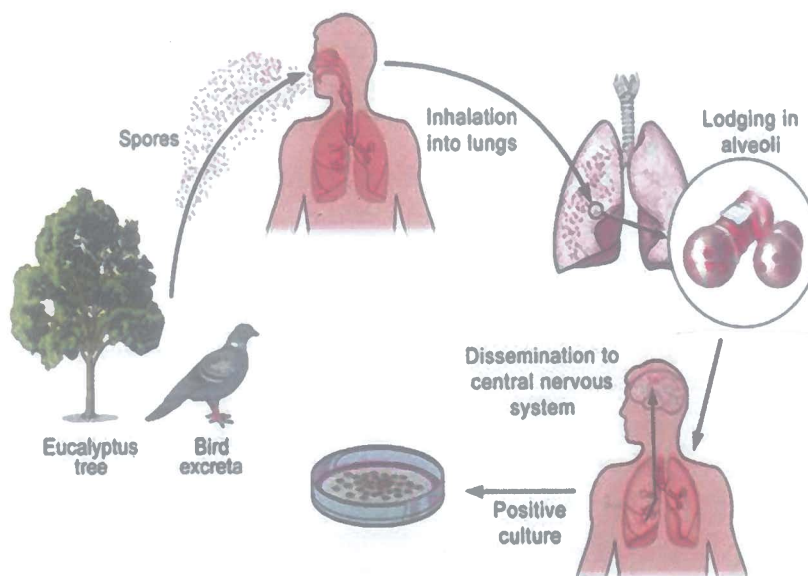
- Invasive infections, e.g. pneumonia or meningitis may occur in severely immunocompromised patients especially those with neutropenia.
- Mycotoxicosis: Aflatoxin of *A. flavus* ingested with spoiled grains or peanut is hepatotoxic and carcinogenic (Fig. 79).



Fig. (79): Mycotoxicosis (Aflatoxin)

## Cryptococcosis

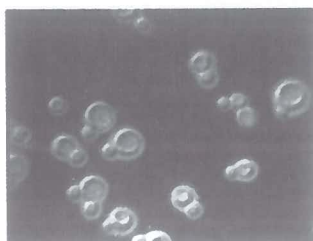
- It is caused by *Cryptococcus neoformans*.
- The organism is present in the soil contaminated with excreta of birds particularly pigeons.
- Infection occurs by inhalation which may result in cryptococcus pneumonia. Spread to the central nervous system leads to **meningitis** (Fig. 80).
- Reduced cell-mediated immunity, especially in AIDS patients, predisposes to severe disease.



**Fig. (80): Pathogenesis of Cryptococcosis**

### Laboratory diagnosis

1. **Specimens:** Sputum or CSF.
2. **Direct detection:**
  - A smear stained with India ink will demonstrate *Cryptococcus neoformans* as budding yeast cells surrounded by a **large gelatinous capsule** (Fig. 81).
  - Detection of capsular antigen in the CSF, by latex agglutination test.
3. **Culture:** on SDA, the organism gives mucoid colonies (Fig. 82).
4. **Identification:** by characteristic morphology and urease production.



**Fig. (81): *Cryptococcus neoformans***  
(India ink stain)



**Fig. (82): *C. neoformans* on SDA**



## ***Pneumocystis***

- ***Pneumocystis jiroveci*** (formerly *P. carinii*) is classified as a yeast on the basis of molecular analysis, but medically many still think of it as a protozoan.
- Most people are infected with *P. jiroveci* by the age of four and develop no symptoms, unless they are immunocompromised.
- It causes **interstitial pneumonia** especially in association with HIV infection.
- The organism cannot be cultivated. It can be detected in clinical specimens (e.g. lung biopsy) after staining with silver stain (Fig. 83).

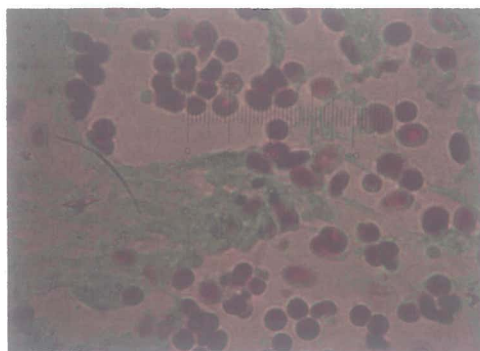


Fig. (83): *Pneumocystis carinii* (Silver stain)

## **Mycetoma (Madura Foot or Maduromycosis)**

- Mycetoma is a localized infection that involves cutaneous and subcutaneous tissue, fascia and bone. It usually affects the foot and rarely the hands and buttocks.
- It is a clinical syndrome characterized by granuloma, multiple abscesses, draining sinuses and pus with granules.
- The organisms involved are present in the soil and are implanted, by trauma, into the tissues especially in bare footed people. Therefore, lesions are localized at the site of the trauma.
- According to causative organisms there are two types of mycetoma (Table 13):
  1. **Eumycotic** mycetoma (caused by true fungi) (Fig. 84)
  2. **Actinomycotic** mycetoma (caused by Actinomycetes) (Fig. 85)



Fig. (84): Eumycotic mycetoma



Fig. (85): Actinomycotic mycetoma

It is important to know whether the mycetoma is caused by fungi or actinomycetes because actinomycotic mycetoma will respond to antibiotics while fungal infection will not. The latter needs surgical treatment up to amputation.

**Table (13):** Eumycotic (fungal) and actinomycotic (bacterial) mycetoma

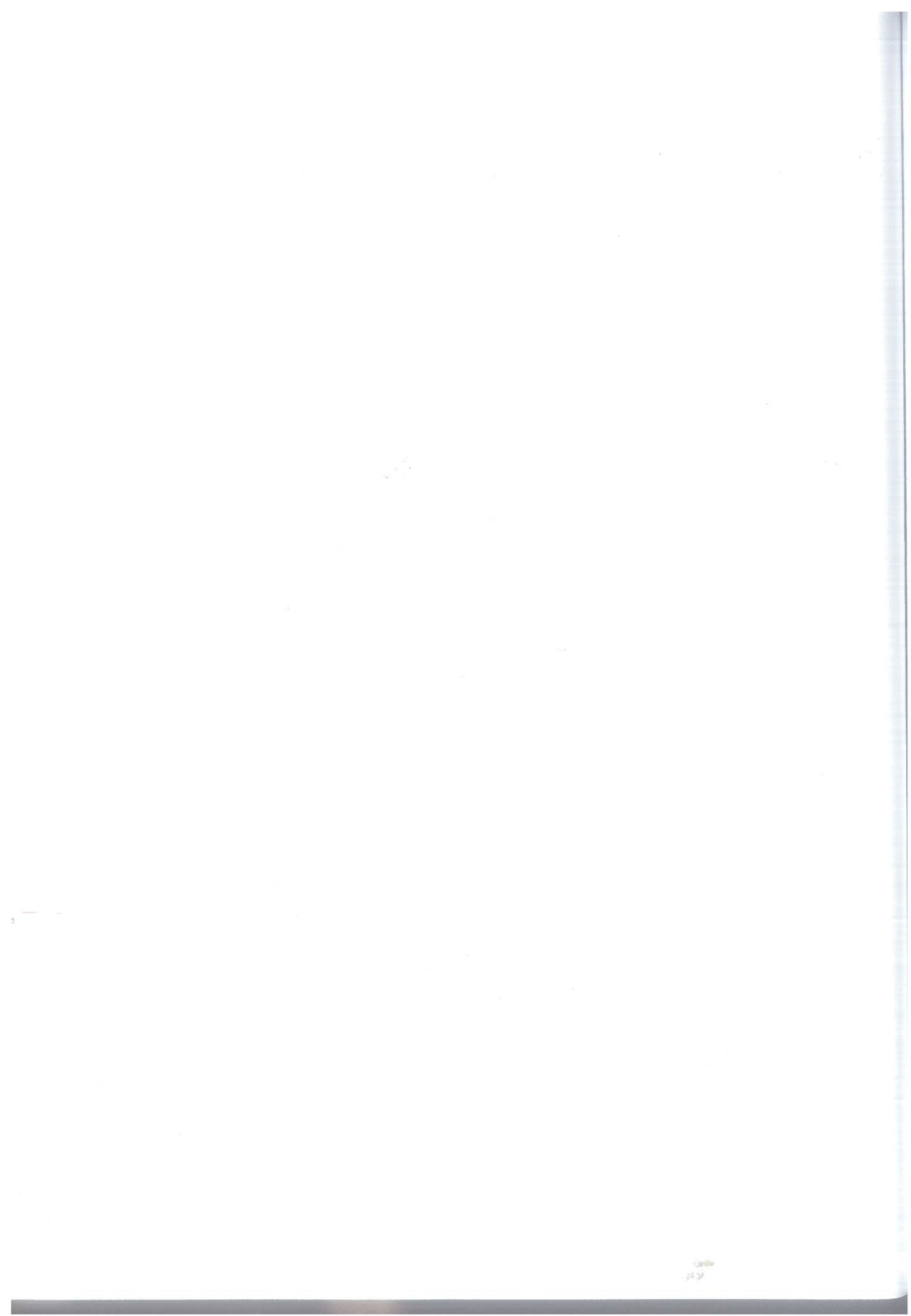
	<b>Eumycotic mycetoma</b>	<b>Actinomycotic mycetoma</b>
<b>Causative agent</b>	Fungal: e.g. <i>Madurella mycetomatis</i> , <i>Madurella grisea</i> and <i>Allescheria boydii</i>	Bacterial: e.g., <i>Actinomadura madurae</i> , <i>Nocardia brasiliensis</i> and <i>Streptomyces somaliensis</i>
<b>Colour of granules</b>	Mainly black and white	Mainly yellow (sulphur)
<b>Microscopic exam</b>	Thick hyphae and spores	Thin fragmented filaments
<b>Culture</b>	- On SDA - aerobic - at 25 - 30°C	- On blood agar - aerobic and anaerobic - at 37°C
<b>Chemotherapy</b>	- No or poor response - Needs surgical treatment and may be amputation	Effective

### MCQs:

- 1- Which medium would be recommended for culturing a CSF specimen for fungi:
  - a- Loeffler's serum medium
  - b- Blood agar
  - c- Modified Thayer-Martin medium
  - d- Sabouraud's dextrose agar
  - e- Lowenstein-Jensen medium
  
- 2- All of the following is correct about *Candida albicans* EXCEPT:
  - a- It causes oral thrush.
  - b- It is a yeast fungus that replicates by budding.
  - c- It produces germ tube in serum at 37°C after 2 hours.
  - d- It produces chlamydospores on corn meal agar.
  - e- It produces septate hyphae at 22°C.
  
- 3- *Cryptococcus neoformans* has the following properties EXCEPT:
  - a- The organism is present in the soil contaminated with excreta of birds.
  - b- Infection occurs by inhalation resulting in cryptococcal pneumonia.
  - c- The organism has a thick capsule surrounding the budding yeast cell.
  - d- Secreted aflatoxin is hepatotoxic and carcinogenic.
  - e- In cases of meningitis, capsular antigen can be detected in CSF.

# **APPLIED MICROBIOLOGY**





## NORMAL FLORA OF THE HUMAN BODY

### ILOs:

By the end of this chapter, the student should be able to:

- Define normal flora
  - Distinguish between normal flora and transient colonization
  - List normal flora of the skin, nose, mouth and throat, colon and vagina
  - Describe the beneficial role of normal flora
  - Describe the harmful effect of normal flora
  - State the conditions where normal flora may produce disease
- 
- Normal flora denotes the population of microorganisms (mainly bacteria) that permanently inhabit the skin and mucous membranes of healthy normal people. They are also termed commensals.
  - Relatively fixed types of organisms are found in a certain site at a given age.
  - Under normal circumstances they are harmless.
  - They are not removed during hand washing with soap and water, but can be partially killed or inhibited by antimicrobial agents.
  - If disturbed, they reestablish themselves.
  - Some people can be transiently colonized with certain organisms derived from the environment, but those are not considered members of the normal flora. They are easily removed by hand washing with soap and water, but do not reestablish themselves.

### Beneficial functions of normal flora

1. They serve nutritional function e.g., intestinal bacteria synthesize vitamin K and vitamin B.
2. They have a protective function through the following:
  - Competition with pathogenic organisms for receptors on host cells.
  - Production of antimicrobial substances e.g., bacteriocin.
  - Maintaining conditions unfavourable for growth of pathogenic organisms e.g., acidic pH maintained by vaginal lactobacilli.

### Harmful effect of normal flora

Normal flora may cause infections under certain conditions:

1. Changes of their normal habitat into a sterile site, e.g.,:
  - a- Subacute endocarditis caused by viridans streptococci.
  - b- Urinary tract infection caused by *E. coli*.
  - c- Peritonitis caused by *B. fragilis* following intestinal perforation.

2. Disturbance of the normal flora, e.g. superinfection caused by *C. difficile* following antibiotic therapy.
3. Lowered host defense mechanisms e.g., in diabetic patients

**Table (14):** Medically important members of the normal flora

Site	Important members	Less important members
Skin	<i>S. epidermidis</i>	<i>S. aureus</i> Diphtheroids Various streptococci <i>Propionibacterium</i> <i>Candida albicans</i>
Nose	<i>S. aureus</i>	<i>S. epidermidis</i> Diphtheroids Viridans streptococci
Mouth and throat	Viridans streptococci	<i>Neisseria</i> <i>S. pyogenes</i> <i>S. pneumoniae</i> <i>Haemophilus influenzae</i> <i>Candida albicans</i> Anaerobes (e.g., <i>Actinomyces</i> and <i>Fusobacteria</i> )
Colon	<i>Bacteroides</i> (predominant) <i>E. coli</i>	<i>Enterococcus</i> <i>Clostridium</i> <i>Lactobacillus</i>
Vagina	<i>Lactobacillus</i>	<i>S. agalactiae</i> <i>Gardnerella vaginalis</i> <i>Candida albicans</i>

**N.B.:**

- Blood and internal organs are generally sterile.
- The stomach contains few organisms because of its low pH and gastric enzymes.

**MCQs:**

- 1- Regarding members of the normal flora, all of the following statements are true **EXCEPT**:
  - a- Transient colonization can be promptly reestablished when disturbed.
  - b- They may cause infections if they change their normal habitat.
  - c- Through "bacterial interference", they prevent colonization of pathogens.
  - d- They may produce disease by superinfection mechanism.
  - e- They synthesize vitamin K in the intestine.
- 2- Regarding the important members of normal flora at different body sites, which of the following pairs is **mis-matched**?
  - a- Skin.....*S. epidermidis*
  - b- Nose.....*S. aureus*
  - c- Throat.....Viridans streptococci
  - d- Colon.....*Bacteroides*
  - e- Vagina.....*E. coli*



## ANAEROBIC INFECTIONS

### ILOs:

By the end of this chapter, the student should be able to:

- Define obligate anaerobes
  - Classify anaerobes
  - List important non-spore forming anaerobic bacteria
  - List the common types of anaerobic infections
  - Outline the diagnosis of anaerobic infections
  - Outline the treatment of anaerobic infections
- 
- The obligate anaerobes are bacteria which can grow **only** in complete absence of oxygen.
  - They are usually classified as:
    - Spore-forming anaerobes which include the genus *Clostridium*.
    - Non-spore-forming anaerobes e.g. *Bacteroides*, *Fusobacterium*, *Lactobacillus*, *Propionibacterium* and *Actinomyces*.
  - Many of the medically important anaerobes form the major part of the normal human flora in the intestine, mouth and female genital tract. They can cause diseases when they leave these sites (endogenous infections).

### Common types of anaerobic infections

1. **Respiratory tract infections:** e.g., Vincent angina, actinomycosis and lung abscess.
2. **Central nervous system infections:** e.g., brain abscess.
3. **Intra-abdominal and pelvic infections:** e.g., appendicitis, liver abscess, peritonitis and pelvic actinomycosis associated with intrauterine device.
4. **Wounds and soft tissue infections:** e.g., gas gangrene.

### Laboratory diagnosis

- **Sample collection:** When anaerobic infection is suspected, it is important that:
  - Specimens are preferably taken before antibiotic therapy.
  - The sample should be taken as deep as possible, away from atmospheric oxygen and as much sample as possible.
  - The sample is taken by a disposable closed syringe or on a swab kept in a reduced transport medium.
  - Specimens are transported as soon as possible to the laboratory.
- The sample is then cultured and incubated under anaerobic conditions for 2-5 days. Colonies are then identified morphologically, biochemically and by gas-liquid chromatography.

## Treatment of anaerobic infections

### 1. Surgical treatment:

Drainage of pus from abscesses, debridement, and removal of necrotic tissues may be sufficient.

### 2. Antimicrobial therapy:

Drugs commonly used to treat anaerobic infections are metronidazole, clindamycin, lincomycin and chloramphenicol. Penicillin may also be used (except in case of *Bacteroides fragilis* which produces  $\beta$ -lactamase).

### 3. In a mixed infection, treatment of the aerobic organisms accompanying anaerobes is also necessary.

## MCQs:

### 1- One of the following organisms is a spore-forming anaerobe:

- a- *Lactobacilli*
- b- *Bacteroides*
- c- *Clostridium*
- d- *Fusobacterium*
- e- *Actinomyces*

### 2- Anaerobes include all of the following EXCEPT:

- a- *Clostridium*
- b- *Treponema*
- c- *Bacteroides*
- d- *Neisseria*
- e- *Fusobacterium*

### 3- True or false:

- a- Obligate anaerobes grow better in absence of oxygen.
- b- Many of the medically important anaerobes can cause endogenous infections.
- c- When anaerobic infection is suspected, the sample should be:
  - i- Taken after initiation of antibiotic therapy that kills aerobic organisms
  - ii- Taken as deep as possible away from atmospheric oxygen
  - iii- Taken as much as possible
  - iv- Taken in a closed test tube or a closed container
  - v- Transported to the laboratory within 2-5 days
  - vi- Kept refrigerated for 2-5 days
  - vii- Cultured and incubated anaerobically for 2-5 days

## IMPORTANT CLINICAL INFECTIOUS DISEASES

### **ILOs:**

**By the end of this chapter, the student should be able to:**

- Define meningitis
- Distinguish between septic and aseptic meningitis
- List the causes of bacterial, viral and fungal meningitis
- Outline the laboratory diagnosis of meningitis
  
- Describe the pathogenesis of urinary tract infection
- State the predisposing factors to urinary tract infection
- List the microorganisms causing urinary tract infection
- Outline the laboratory diagnosis of urinary tract infection
- Define significant bacteriuria
- Define and list the causes of sterile pyuria
- List the categories of urethritis
  
- Define and list the causes of noninvasive gastroenteritis
- Define and list the causes of invasive gastroenteritis
- Identify and compare between common types of food poisoning
- List the causes of hepatitis
  
- List the microbial causes of upper respiratory tract infections
- List the microbial causes of acute otitis media and otitis externa
- Identify the types of lower respiratory tract infections
- List the causes of pneumonia
- List the causes of health-care associated pneumonia
- List the causes of atypical pneumonia
- Outline the laboratory diagnosis of pneumonia
  
- List the microbial causes of eye infections
  
- Define pyrexia of undetermined origin
- List the major causes of pyrexia of undetermined origin
- List the infective causes of pyrexia of undetermined origin
- Outline the diagnosis of pyrexia of undetermined origin



## Meningitis

Meningitis is the infection of the membranes surrounding the brain and spinal cord.

- There are two major forms of meningitis:
    - **Septic meningitis** typically caused by bacteria.
    - **Aseptic meningitis** caused by viruses, fungi, *Mycobacterium tuberculosis* or spirochaetes.
  - Bacterial meningitis can be severe and may result in brain damage, hearing loss, or learning disability. Thus bacterial meningitis is a medical emergency that indicates prompt initiation of antibiotic treatment.
- Viral meningitis is generally less severe and resolves without specific treatment.

### Causative organisms

#### 1. Bacterial causes

- **Neonatal meningitis**
  - *Streptococcus agalactiae*
  - *Escherichia coli* (usually having K1 antigen), and other Gram-negative enteric bacilli e.g., *K. pneumoniae*
  - *Listeria monocytogenes*
- **Children and adults meningitis**
  - **Common causes**
    - *Neisseria meningitidis*
    - *Streptococcus pneumoniae*
    - *Haemophilus influenzae* type b
  - **Less common causes**
    - *Mycobacterium tuberculosis*
    - *Listeria monocytogenes*
    - *Staphylococcus* species
    - *Streptococcus* species
    - Gram-negative enteric bacilli
    - *Pseudomonas aeruginosa*
    - Spirochaetes:
      - *Leptospira interrogans* (Weil's disease)
      - *Treponema pallidum* (syphilitic meningitis in secondary syphilis and neurosyphilis in tertiary syphilis)
      - *Borrelia burgdorferi* (Lyme disease)

#### 2. Viral meningitis

- Enteroviruses: Coxsackieviruses A and B, echoviruses and polioviruses
- Herpes simplex virus type 2 (HSV-2)
- Adenoviruses

#### 3. Fungal meningitis

- *Cryptococcus neoformans*
- *Coccidioides immitis*

## Laboratory diagnosis of meningitis

### A- Specimens:

- CSF should be collected, by lumbar puncture, before initiation of antibiotic therapy and under strict aseptic precautions.
- Blood specimens may be used for blood culture since bacteraemia is common in septic meningitis.

### B- CSF analysis:

Examination of uncentrifuged CSF allows differentiation between septic and aseptic meningitis as shown in table (15)

**Table (15):** The CSF findings in septic and aseptic meningitis

Diagnosis	Aspect	Pressure (mmH <sub>2</sub> O)	Leucocytes per ml	Predominant leucocytes	Glucose mg/dl	Protein mg/dl
Normal	Clear	< 200	0-5	Lymphocytes	45-85	15-45
Septic (bacterial) meningitis	Cloudy	Increased	200-20,000	Neutrophils	Low	High
Aseptic (viral) meningitis	Clear	Slightly increased	100-1000	Lymphocytes	Normal or low	Moderately high

### N.B.

- CSF findings in tuberculous and fungal meningitis are the same as septic meningitis except cytologically where lymphocytes predominate and do not exceed 1000/ml.
- In case of tuberculous meningitis, a fibrin web may develop on standing.

### C- Direct detection

#### • Microscopic examination

1. Gram-stained smears prepared from sediment of centrifuged CSF may reveal:
  - Gram-negative intracellular diplococci (*N. meningitidis*)
  - Gram-negative coccobacilli (*H. influenzae*)
  - Gram-positive diplococci (*S. pneumoniae*)
2. Ziehl-Neelsen stained smears to detect AFB.
3. Fresh unstained smears examined by dark ground microscopy may reveal motile spirochaetes.
4. India ink stained smears in suspected cryptococcosis.

- **Direct antigen detection**

Latex agglutination test may be used to detect *N. meningitidis*, *H. influenzae* and *S. pneumoniae* capsular antigens in CSF.

- **Molecular techniques**

PCR allows the rapid diagnosis of some viral agents.

Results of direct detection tests must be reported within one hour to the treating physician to direct the antimicrobial therapy.

#### **D- Cultivation**

- **CSF deposit** is plated on:

- Chocolate and blood agar in septic meningitis
- Lowenstein-Jensen medium in tuberculous meningitis
- Sabouraud's agar in fungal meningitis
- Cell cultures in suspected viral meningitis

- **Blood samples** should be cultivated by the blood culture technique. Subcultures are plated on chocolate and blood agar.

**E- Identification** of cultures is carried out in a systematic way according to suspected organism.

#### **F- Serology:**

- Serological diagnosis of viral causes can be achieved by demonstrating IgM antibody or fourfold increase of IgG in paired sera.
- Serum CRP may differentiate bacterial from viral meningitis. It is positive in bacterial and negative in viral meningitis.



## Urinary Tract Infections

- The urinary tract is one of the most common sites of bacterial infections, particularly in females.
- Organisms from the faecal flora usually enter the urinary tract by ascending from the perineum and peri-urethral sites (**ascending infection**). Rarely, the kidney can be infected by the haematogenous route.

### Predisposing factors:

1. Short female urethra.
2. Sexual intercourse.
3. Senile prostatic hypertrophy.
4. Structural and neurological abnormalities of the urinary tract which are associated with residual urine, i.e. incomplete emptying of the bladder after micturition.
5. Instrumentation, e.g. urinary catheters which are the most important predisposing cause of UTI in hospitalized patients.
6. Other host factors as pregnancy, diabetes mellitus and immunosuppression.

### Causative organisms:

- *Escherichia coli* is the commonest cause. It accounts for up to 80% of community-acquired cases and about 50% of healthcare-associated cases.
- Other Gram-negative bacilli include *Klebsiella*, *Proteus* and *Pseudomonas* species.
- Gram positive cocci include staphylococci (especially *Staphylococcus saprophyticus*) and *Enterococcus faecalis*.
- *Mycobacterium tuberculosis*.
- Adenoviruses.
- *Candida albicans*.

## Laboratory diagnosis

### I. Sample collection:

- After adequate cleaning of the external genitalia with tap water and drying, a **mid-stream** specimen of urine is obtained in a sterile container.
- In infants:
  - Adhesive sterile bags may be used.
  - Suprapubic aspiration is occasionally necessary.
- Catheter sample should be avoided unless the patient is already catheterized.
- If urinary tuberculosis is suspected, 5 successive morning samples should be collected on 5 consecutive days.

### II. Transport to the laboratory:

- The urine sample should reach the laboratory within 2 hours of collection.
- If not possible, the sample should be kept at 4°C to avoid multiplication of possible contaminants which may result in a false high bacterial count.

- III. Microscopic examination:** A wet film of urine deposit is examined for the presence of pus cells, RBCs, bilharzial ova, casts, crystals ... etc.

#### IV. Culture:

Urine is normally sterile. Contamination of urine samples may occur by peri-urethral flora during collection. Infection can be distinguished from contamination by viable bacterial count.

- **Viable bacterial count**

- This is done by spreading a measured volume of uncentrifuged urine on the surface of a solid medium. After incubation, colonies are counted.
- A count of  $10^5$  organisms per ml is regarded as "**significant bacteriuria**" (i.e. urinary tract infection).
- The urine is cultured either on CLED agar\* or on blood agar and MacConkey's medium. After incubation at 37°C for 24 hours, the growing colonies are identified in a systematic way.
- When tuberculosis is suspected, urine samples are cultured on L.J. medium.

#### Sterile pyuria:

It means absence of growth on ordinary culture media despite the presence of pus cells in urine (more than 10/HPF). This may be due to:

1. Recent antimicrobial chemotherapy
2. Renal tuberculosis
3. Female genital tract infections
4. Non-gonococcal urethritis (e.g., *Chlamydia trachomatis*) in male patients
5. Prostatitis
6. Non-infectious conditions: Neoplasia of the renal tract, renal calculi, catheterization and fever in children irrespective of the cause

## Urethritis

Urethritis is an inflammatory condition that can be infectious or non-infectious. Infectious causes of urethritis are typically sexually transmitted, but post-traumatic infections following catheterization or instrumentation may also occur.

#### I. Sexually transmitted urethritis:

1. **Gonococcal urethritis** due to *Neisseria gonorrhoeae*
2. **Non-gonococcal urethritis** may be due to:
  - *Chlamydia trachomatis* (serotypes D-K).
  - Genital mycoplasma (*M. genitalium*, *M. hominis* and *Ureaplasma urealyticum*).
  - *Trichomonas vaginalis*.

#### II. Post-traumatic urethritis: may be due to *E. coli*, staphylococci and streptococci.

---

\* CLED agar (cystine lactose electrolyte-deficient agar) allows the growth of both Gram negative and Gram positive pathogens. The pH indicator in CLED agar is bromothymol blue and, therefore, lactose-fermenting colonies appear yellow. This medium is electrolyte-deficient to prevent the swarming of *Proteus* species.

## Gastroenteritis (Diarrhoea)

Diarrhoea can be categorized as non-inflammatory and inflammatory (table 16):

**Table (16):** Comparison between non-inflammatory and inflammatory gastroenteritis:

	Non-inflammatory (non-invasive) gastroenteritis	Inflammatory (invasive) gastroenteritis (dysentery)
Pathogenesis	Enterotoxin production	Mucosal invasion and destruction
Site	Small intestine	Large intestine
Fever	No	Present
Stools	<ul style="list-style-type: none"> <li>- Watery</li> <li>- Large volume</li> <li>- No RBCs or WBCs</li> </ul>	<ul style="list-style-type: none"> <li>- Bloody</li> <li>- Small volume</li> <li>- Many RBCs and WBCs</li> </ul>
Causative organisms	<ul style="list-style-type: none"> <li>- <i>Enterotoxigenic E. coli</i></li> <li>- <i>Vibrio cholerae</i></li> <li>- <i>Clostridium perfringens</i> (food poisoning)</li> <li>- <i>Bacillus cereus</i> (food poisoning)</li> <li>- Viral causes include: <ul style="list-style-type: none"> <li>▪ Rota virus</li> <li>▪ Noro virus</li> <li>▪ Adenovirus</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- <i>Shigella</i> species</li> <li>- <i>S. Enteritidis</i> &amp; <i>S. Typhimurium</i> (food poisoning)</li> <li>- <i>Campylobacter jejuni</i></li> <li>- Enteroinvasive <i>E. Coli</i></li> <li>- Enterohemorrhagic <i>E. coli</i> (<i>E. coli</i> O157:H7)</li> <li>- <i>Clostridium difficile</i></li> <li>- <i>Yersinia enterocolitica</i>.</li> </ul>

## Food Poisoning

- Food poisoning is defined as an outbreak in which two or more individuals experience a similar illness resulting from the ingestion of the same food.
- Patients with food poisoning typically present with gastrointestinal tract symptoms (vomiting, diarrhoea and abdominal pain). However, other presentations may occur which help recognizing the aetiologic agents.
- Common types of food poisoning are summarized in table 17:



Table (17): Comparison of common types of food poisoning

Causative organism	Pathogenesis	Associated foods	Incubation period	Signs and symptoms
<b><i>Staphylococcus aureus</i></b> (the most common)	Preformed enterotoxin	- Carbohydrate rich food e.g. cake - Protein rich food e.g. milk and its products	1- 6 hrs	- Severe vomiting - Diarrhoea - No fever
<b><i>S. Enteritidis</i> &amp; <i>S. Typhimurium</i></b>	Invasion of intestinal epithelium (no toxin)	- Raw eggs - Poultry	8-48 hrs	- Fever - Severe diarrhoea - Abdominal cramps
<b><i>Clostridium perfringens</i></b>	Release of enterotoxin in gut	- Cooked meat and meat products	8-24 hrs	- Diarrhoea - Abdominal cramps
<b><i>Clostridium botulinum</i></b>	Preformed neurotoxin	- Canned food - Salted fish	12-36 hrs	- Diplopia - Dysphagia - Difficulty in breathing - Death
<b><i>Bacillus cereus</i></b>	a. Emetic form: a preformed heat-stable enterotoxin	- Fried rice	1-6 hrs	- Vomiting - Abdominal cramps
	b. Diarrhoeal form: a preformed heat-labile enterotoxin	- Meat	6-24 hrs	- Diarrhoea - Abdominal cramps
<b><i>Listeria monocytogenes</i></b>	Invasion of intestinal epithelium	- Fresh soft cheese - Undercooked meat	8-48 hrs	- Diarrhoea - Abdominal cramps - Fever

N.B.:

- In case of food poisoning, fluid and electrolytes may be needed.
- Spontaneous recovery is the rule except in:
  - Salmonella food poisoning: Antibiotic therapy may be required.
  - Botulism: Antitoxin should be administered within 12 hours.

## Hepatitis

Microbial causes of hepatitis can be classified as:

### A) Viral:

1. Viruses infecting the liver as a primary target include hepatitis viruses (HAV, HBV, HCV, HDV, and HEV).
2. Viruses infecting the liver as a secondary target include yellow fever virus, Epstein-Barr virus and cytomegalovirus.

### B) Bacterial:

1. Q fever (*Coxiella burnetii*).
2. Leptospirosis (*Leptospira interrogans*).

### C) Fungal:

1. Aflatoxin poisoning: *Aspergillus flavus* mycotoxin.
2. Ergot poisoning

## Respiratory Tract Infections

The nose is the main portal of entry for microorganisms to the respiratory system.

### I. Upper respiratory tract infections

**Table (18):** Causative organisms of upper respiratory tract infections:

Infection	Bacterial causes	Viral causes
Pharyngitis (70% of cases are caused by viruses)	- <i>S. pyogenes</i> - Vincent's angina - <i>C. diphtheria</i>	- Rhinoviruses - Coronaviruses - Adenoviruses - Influenza virus - Parainfluenza virus - Herpes viruses
Epiglottitis	- <i>H. influenzae</i>	—
Acute otitis media and acute sinusitis	- <i>S. pneumoniae</i> - <i>H. influenzae</i> - <i>Moraxella catarrhalis</i>	- Respiratory syncytial virus - Adenoviruses
Otitis externa	- <i>S. aureus</i> - <i>P. aeruginosa</i>	—

#### N.B.:

- *C. albicans* may cause oral thrush (pharyngitis) as superinfection.
- Anaerobic bacteria and fungi may cause sinusitis.

### II. Lower respiratory tract infections

- They include tracheitis, bronchitis, bronchiolitis, pneumonia and pleurisy.
- These infections tend to be more severe than infections of the upper respiratory tract and the choice of appropriate antimicrobial therapy may be life-saving.

#### Causes of pneumonia

- **Bacterial pneumonia**
  - *Streptococcus pneumoniae* (the most common bacterial cause)
  - *Haemophilus influenzae*
  - *Klebsiella pneumoniae*
  - *Staphylococcus aureus*
  - *Mycoplasma pneumoniae*
  - *Chlamydia species*
  - *Legionella pneumophila*
  - *Coxiella burnetii*
  - *Pseudomonas aeruginosa*
  - *Acinetobacter species*
  - *Mycobacterium tuberculosis*

- **Viral pneumonia**

- Influenza viruses
- Parainfluenza viruses
- Respiratory syncytial virus

- **Fungal pneumonia**

- *Pneumocystis jiroveci*
- *Histoplasma capsulatum*

**N.B.:**

- Viral infections may be complicated by secondary bacterial infections (especially by *S. aureus*).
- Common pathogens in healthcare-associated pneumonia include:
  - *Klebsiella pneumoniae*
  - *S. aureus* (including MRSA)
  - *Pseudomonas aeruginosa*
  - *Acinetobacter* species
- *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Chlamydia* species, *Coxiella burnetii*, viruses and fungi cause a type of pneumonia clinically designated as **atypical pneumonia** which is characterized by:
  - Low-grade fever
  - Dry cough with scanty sputum
  - Failure to demonstrate the causative organism by Gram stain or its isolation on ordinary culture media

**Laboratory diagnosis**

- Specimens include sputum (preferably morning samples), broncho-alveolar lavage (BAL) or blood.
- Sputum smears must be first examined by Gram stain to determine that it is not simply saliva but truly sputum (many neutrophils and few epithelial cells).
- Acceptable specimens are then subjected to the different methods of diagnosis.
- Blood cultures may be helpful in diagnosis because bacterial pneumonia is often associated with bacteraemia.



## Eye infections

**Table (19):** Microbial causes of eye infections:

	Organism	Disease
<b>Bacterial</b>	<i>Chlamydia trachomatis</i> (serotypes A-C)	- Trachoma
	<i>Chlamydia trachomatis</i> (serotypes D-K)	- Inclusion conjunctivitis
	<i>Neisseria gonorrhoeae</i>	- Ophthalmia neonatorum
	<i>Streptococcus pneumoniae</i>	- Conjunctivitis (not highly Communicable)
	<i>Haemophilus aegyptius</i>	- Acute mucopurulent conjunctivitis (pink eye)
	<i>Staphylococcus aureus</i>	- Sticky eye in neonates - Eyelid infection (styes)
	<i>Pseudomonas aeruginosa</i>	- Keratitis - Endophthalmitis (following trauma)
<b>Viral</b>	Adenoviruses	- Swimming pool conjunctivitis (pink eye) - Follicular conjunctivitis - Acute haemorrhagic conjunctivitis - Epidemic keratoconjunctivitis
	Herpes simplex virus-1	- Keratoconjunctivitis - Corneal ulcers
	Measles virus	- Conjunctivitis
<b>Fungal (rare)</b>	<i>Candida albicans</i>	- Corneal ulcers
	<i>Aspergillus species</i>	

## Pyrexia of Undetermined Origin

A patient who presents with pyrexia as a predominant clinical feature, for at least 10 days, without an obvious cause is considered as a case of pyrexia of undetermined origin (PUO). Infective causes are responsible for 75% of cases of acute PUO.

### Infective causes of PUO

#### A- Non-specific causes:

1. Cryptic abscesses in abdomen and pelvis
2. Infective endocarditis
3. Urinary tract infections
4. Ear, sinus or dental infections

**B- Specific causes:** Some specific microbial causes are given in table 20:

**Table (20):** Specific microbial causes of PUO:

<b>Bacterial diseases</b>	<ul style="list-style-type: none"><li>- Typhoid</li><li>- Brucellosis</li><li>- Tuberculosis</li><li>- Typhus</li><li>- Leptospirosis</li><li>- Relapsing fever</li><li>- Q fever</li></ul>
<b>Viral diseases</b>	<ul style="list-style-type: none"><li>- Hepatitis A or B</li><li>- Cytomegalovirus disease</li><li>- Infectious mononucleosis</li><li>- HIV infection (AIDS)</li><li>- Yellow fever</li></ul>
<b>Fungal diseases</b>	<ul style="list-style-type: none"><li>- Deep fungal infections, e.g.:<ul style="list-style-type: none"><li>▪ Cryptococcosis</li><li>▪ Systemic candidiasis</li></ul></li></ul>

### Laboratory diagnosis of PUO

- Blood culture
- Urine analysis and culture
- Stools analysis and culture
- Serological tests: e.g.,
  - Widal test (typhoid fever)
  - ASO test (rheumatic fever)
  - Brucella agglutination test (brucellosis)
  - Paul-Bunnell or monospot test (infectious mononucleosis)
  - Western blot test (HIV infection)

**MCQs:**

- 1- Neonatal meningitis is frequently caused by:
  - a- Enteroinvasive *E. coli*
  - b- *E. coli* K1
  - c- *E. coli* O157:H7
  - d- *K. rhinoscleromatis*
  - e- *K. ozaenae*
- 2- One of the causes of sterile pyuria is:
  - a- Renal tuberculosis
  - b- Urine contamination
  - c- Antibiotic-resistant bacteria
  - d- Bacteriocin-producing *E. coli*
  - e- Appropriate methods of sterilization
- 3- Each of the following agents is a recognized cause of diarrhoea **EXCEPT**:
  - a- *Clostridium perfringens*
  - b- *Enterococcus faecalis*
  - c- *Shigella dysenteriae*
  - d- *Vibrio cholerae*
  - e- *Campylobacter jejuni*
- 4- Food poisoning is caused by the following **EXCEPT**:
  - a- *Clostridium botulinum*
  - b- *Clostridium perfringens*
  - c- *Bacillus cereus*
  - d- *Salmonella* Typhi
  - e- *Staphylococcus aureus*
- 5- The following organisms cause primary atypical pneumonia **EXCEPT**:
  - a- *Chlamydophila pneumoniae*
  - b- *Mycobacterium tuberculosis*
  - c- *Legionella pneumophila*
  - d- *Mycoplasma pneumoniae*
  - e- *Coxiella burnetii*
- 6- Pyrexia of undetermined origin (PUO) may be due to any of the following bacterial diseases **EXCEPT**:
  - a- Tuberculosis
  - b- Typhoid
  - c- Leptospirosis
  - d- Rift valley fever
  - e- Brucellosis



## TRANSMISSION-BASED INFECTIONS

### **ILOs:**

**By the end of this chapter, the student should be able to:**

- List the most common causes of sexually transmitted diseases
- Define pre-natal (congenital) infections
- List the causes of pre-natal (congenital) infections
- Define peri-natal infections
- List the causes of peri-natal infections
- Outline the diagnosis of diseases transmitted from mother to foetus
- Define zoonotic diseases
- Outline the routes of transmission of zoonotic diseases
- List the most common causes of zoonotic diseases
- List pathogens transmitted by milk and milk products
- List the microorganisms transmitted by blood transfusion

## Sexually Transmitted Diseases

The most common sexually transmitted diseases (STDs) are summarized in table 21:

**Table (21):** Common sexually transmitted diseases:

	Organism	Disease
<b>Bacterial causes:</b>	<i>Neisseria gonorrhoeae</i>	Gonorrhoea
	<i>Treponema pallidum</i>	Syphilis
	<i>Haemophilus ducrey</i>	Chancroid (soft sore)
	<i>Chlamydia trachomatis</i> (serotypes L1, 2 and 3)	Lymphogranuloma venereum
	<i>Chlamydia trachomatis</i> (serotypes D-K)	Nongonococcal urethritis
	<i>Ureaplasma urealyticum</i>	Nongonococcal urethritis
<b>Viral causes:</b>	Hepatitis B virus	Viral B hepatitis
	Human immunodeficiency virus	Acquired immunodeficiency syndrome (AIDS)
	Herpes simplex virus-2	Herpes genitalis
	Human papillomaviruses	Genital warts & cervical cancer
	Molluscum contagiosum Virus	Genital warts
	Cytomegalovirus	Mononucleosis-like syndrome
	Adenoviruses	Orchitis, cervicitis, urethritis & genital ulcers

**N.B.:**

1. The patient may have more than one STD at the same time.
2. The causative organisms are delicate and die quickly outside the body.
3. Not all sexually transmitted diseases are venereal diseases (e.g., HBV and HIV are transmitted sexually but they do not cause genital lesions).

## Diseases Transmitted from Mother to Foetus

Infections transmitted from mother to foetus may occur either during pregnancy (prenatal) or during delivery through an infected birth canal (perinatal) (Table 22a and 22b):

**Table (22a): Prenatal infections:**

	Organism	Disease
<b>Viral causes</b>	<ul style="list-style-type: none"> <li>- Rubella virus</li> <li>- Cytomegalovirus</li> <li>- Varicella zoster virus</li> <li>- HIV</li> <li>- Parvovirus B19</li> <li>- Zika virus</li> </ul>	<ul style="list-style-type: none"> <li>- Congenital rubella syndrome</li> <li>- Cytomegalic inclusion disease</li> <li>- Congenital varicella syndrome</li> <li>- HIV infection</li> <li>- Hydrops foetalis</li> <li>- Microcephaly</li> </ul>
<b>Bacterial causes</b>	<ul style="list-style-type: none"> <li>- <i>Treponema pallidum</i></li> <li>- <i>Listeria monocytogenes</i></li> </ul>	<ul style="list-style-type: none"> <li>- Congenital syphilis</li> <li>- Sepsis with high mortality</li> </ul>
<b>Protozoal causes</b>	<ul style="list-style-type: none"> <li>- <i>Toxoplasma gondii</i></li> </ul>	<ul style="list-style-type: none"> <li>- Congenital toxoplasmosis</li> </ul>

The above mentioned prenatal infections may result in abortion, intrauterine foetal death or premature labour.

**Table (22b): Perinatal infections:**

	Organism	Disease
<b>Viral causes</b>	<ul style="list-style-type: none"> <li>- Hepatitis B virus</li> <li>- Cytomegalovirus</li> <li>- Herpes simplex virus-2</li> <li>- HIV</li> </ul>	<ul style="list-style-type: none"> <li>- Chronic hepatitis</li> <li>- CMV infection</li> <li>- Neonatal herpes</li> <li>- HIV infection</li> </ul>
<b>Bacterial causes</b>	<ul style="list-style-type: none"> <li>- <i>Neisseria gonorrhoeae</i></li> <li>- <i>Chlamydia trachomatis</i></li> <li>- <i>Streptococcus agalactiae</i></li> <li>- <i>Listeria monocytogenes</i></li> </ul>	<ul style="list-style-type: none"> <li>- Ophthalmia neonatorum</li> <li>- Conjunctivitis &amp; pneumonia</li> <li>- Sepsis &amp; meningitis</li> <li>- Sepsis &amp; meningitis</li> </ul>

### Laboratory diagnosis

- Serologic tests are considered the most important tests to confirm clinically suspected infection.
- For the mother, diagnosis of Toxoplasmosis, Rubella, Cytomegalovirus and Herpes virus infections (ToRCH) is done by detection of specific IgM or a 4-fold rise of specific IgG.
- For the newborn, detection of specific IgM indicates intrauterine infection.



## Zoonotic Diseases

- These are human diseases caused by organisms that are acquired from **animals**.
- Man may be end host (no man-man transmission).
- Some occupations have a higher incidence of reservoir contact such as veterinarians, slaughterhouse workers, farmers and butchers.
- Transmission routes include:
  - Direct contact with infected tissues.
  - Ingestion of infectious meat or milk.
  - Inhalation of infectious aerosols.
  - Inoculation by the bite of an infected animal or vector such as mosquitoes, fleas, or ticks.

### The most common zoonotic infections are:

#### 1) Bacterial:

- **Gram-positive bacilli**
  - *Bacillus anthracis* (anthrax)
  - *Listeria monocytogenes* (listeriosis)
- **Gram-negative bacilli**
  - *Brucella* species (brucellosis)
  - *Campylobacter jejuni* (diarrhoea)
  - *Salmonella* Enteritidis (food poisoning)
  - EHEC (diarrhoea)
  - *Yersinia pestis* (bubonic plague)
- **Mycobacteria**
  - *Mycobacterium bovis* (tuberculosis)
- **Spirochaetes**
  - *Borrelia hermsii* (endemic relapsing fever)
  - *Borrelia burgdorferi* (Lyme disease)
  - *Leptospira interrogans* (leptospirosis)
- **Chlamydiae**
  - *Chlamydia psittaci* (psittacosis)
- **Rickettsiae**
  - *Rickettsia typhi* (endemic typhus)
  - *Rickettsia rickettsii* (Rocky Mountain spotted fever)
- **Coxiella**
  - *Coxiella burnetii* (Q fever)

#### 2) Viral:

- Rabies virus (rabies)
- Influenza A virus (avian & swine flu)
- Yellow fever virus (yellow fever)
- Rift valley fever virus (rift valley fever)
- Hantavirus and Ebola viruses (haemorrhagic fever)

## Pathogens Transmitted by Milk and Milk Products

1. Diseased animals may excrete pathogens directly in milk. These pathogens are eradicated by pasteurization. They include:
  - *M. bovis*
  - *Brucella abortus* and *melitensis*
  - *Coxiella burnetii*
2. Contamination of milk may occur from the cow's faeces, skin commensals, infected udder, environment, insects or hands of people handling the milk. Important pathogens include:

### a. Bacteria:

- *Salmonella* Typhi and *S. Paratyphi*
- *Shigella* species
- *Campylobacter* species
- *S. aureus*
- *Listeria monocytogenes*
- EHEC
- *Vibrio cholerae*

### b. Viruses:

- Poliomyelitis virus
- Hepatitis A and E viruses
- Rotavirus

## Microorganisms Transmitted by Blood Transfusion

1. Hepatitis viruses B, C and D.
  2. HIV types 1 and 2.
  3. Cytomegalovirus (CMV).
  4. Epstein-Barr virus (EBV).
  5. Human T-cell lymphotropic virus (HTLV-1).
  6. Human parvovirus B19 (HPV-B19).
  7. *Treponema pallidum*.
- CMV, EBV and HTLV are present in the cellular components of blood, thus, are not transmitted by plasma.
  - *Treponema pallidum* dies when stored at 4°C within 3-5 days, so it is not transmitted by stored blood.
  - Blood may be contaminated by bacteria during withdrawal from the donor or from the environment.

**MCQs:**

- 1- One of the following can be transmitted sexually but does not cause genital lesions:
  - a- *Treponema pallidum*
  - b- Hepatitis B virus
  - c- *Chlamydia trachomatis*
  - d- Herpes simplex virus
  - e- *Haemophilus ducrey*
- 2- Transplacental infections are caused by the following **EXCEPT**:
  - a- Varicella-Zoster virus
  - b- Cytomegalovirus
  - c- Rubella virus
  - d- *Haemophilus ducrey*
  - e- *Treponema pallidum*
- 3- The following organisms have animal reservoirs **EXCEPT**:
  - a- *Brucella melitensis*
  - b- *Yersinia pestis*
  - c- *Bordetella pertussis*
  - d- *Bacillus anthracis*
  - e- *Campylobacter jejuni*
- 4- Pathogens excreted in milk from diseased animals include all the following **EXCEPT**:
  - a- *Mycobacterium bovis*
  - b- *Brucella abortus*
  - c- *Brucella melitensis*
  - d- *Vibrio cholerae*
  - e- *Coxiella burnetii*
- 5- All of the following viruses can be transmitted by blood transfusion **EXCEPT**:
  - a- Cytomegalovirus
  - b- Rubella virus
  - c- Hepatitis viruses B, C & D
  - d- HIV types 1 & 2
  - e- Epstein-Barr virus



## HOSPITAL-ACQUIRED INFECTION

### **ILOs:**

**By the end of this chapter, the student should be able to:**

- Define healthcare-associated infections (HAIs)
  - Recognize factors favouring HAIs
  - Recognize the sources of HAIs
  - List the mode (s) of transmission of HAIs
  - Distinguish droplet from airborne transmission
  - List common types of HAIs
  - Define isolation precautions
  - State the principle of the standard precautions
  - Define transmission-based precautions
  - List the indications and measures of airborne precautions
  - List the indications and measures of droplet precautions
  - List the indications and measures of contact precautions
  - Describe investigations of outbreaks in hospitals
- 
- Hospital-acquired infection is also called healthcare-associated infection (HAI) or nosocomial infection.
  - It is defined as an infection that is acquired by a patient in a hospital (or other health care facility e.g. nursing home or rehabilitation center), and which was not present or incubating at admission. Infections occurring more than 48 hours after admission are usually considered nosocomial.
  - HAIs are a serious problem in the hospital. Therefore, each hospital should have an infection control (IC) program designed to prevent acquisition of infections.

### **Sources of infections**

1. **Endogenous**, where the organism is acquired from the patient's own normal flora
2. **Exogenous**
  - People: whether other infected patients or medical personnel
  - Hospital environment: e.g. instruments, ventilators, bedpans and air condition system
  - Blood and blood products

## Factors favouring healthcare-associated infections

### I. Host factors

- Extreme of age (neonates & elderly)
- Lowered resistance which may be due to an underlying disease e.g. diabetes, malignancy or due to immunosuppressive therapy e.g. in transplant patients.
- Instrumentation e.g. using urinary catheters, ventilators, endoscopes, venous and arterial catheters.

### II. Microbial factors

- The hospital environment harbours highly virulent organisms e.g. *S. aureus*, *E. coli*, *Klebsiella* and *Pseudomonas*. However, opportunistic pathogens may cause infection in immunosuppressed patients.
- The wide use of antibiotics in the hospital favours the development of microbial drug resistance e.g. MRSA and VRSA and VRE.

## Mode of transmission

The infectious agent may be transmitted from the source to a susceptible host by the following modes:

- **Contact**
  - Direct contact by hands of healthcare personnel
  - Indirect contact by contaminated objects e.g. thermometers
- **Droplet**
  - Droplets containing microorganisms are generated from an infected person during coughing, sneezing and talking.
  - These droplets are large in size ( $> 5\mu\text{m}$ ); therefore, they settle down rapidly and, generally, travel only short distance (no more than 1m).
  - Accordingly, transmission requires close contact between the source of infection and a susceptible person.
  - Diseases spread by droplet include influenza, rubella, mumps, pertussis, pneumococcal pneumonia, meningococcal meningitis and many others.
- **Airborne**
  - Droplet nuclei containing microorganisms are generated from evaporated droplets.
  - These droplet nuclei are small in size ( $< 5\mu\text{m}$ ); therefore, they remain suspended in the air for long periods of time and can be carried by air currents on dust particles for long distances.
  - Accordingly, transmission does not necessitate close contact between the source of infection and a susceptible person.
  - Fortunately, only a limited number of diseases are transmitted by this route, namely, tuberculosis, measles, anthrax and chickenpox.
- **Blood or needle prick**
- **Common vehicle**

Transmission may occur by contaminated items such as food, water, medication and instruments.
- **Vector**

The infectious agents may be transmitted through insects e.g. mosquitoes.

## Common types of HAIs

1. Urinary tract infections e.g. by *E. coli*, *Klebsiella*, *Pseudomonas* and *Proteus*
2. Surgical wound infections e.g. by staphylococci, Gram negative bacilli and enterococci
3. Lower respiratory tract infections (pneumonia) e.g. by *S. aureus* and Gram negative bacilli
4. Blood stream infections e.g. staphylococci, Gram negative bacilli and enterococci

## Investigations of outbreaks in hospitals

- Microbiological sampling from patients, health care workers and suspected sources may be indicated when investigating hospital outbreaks e.g. surgical wound infection and MRSA outbreaks in ICUs.
- Isolated organisms are identified to species level followed by typing, either by non-molecular methods (e.g. phage typing) or molecular methods (e.g. plasmid analysis).

## Isolation precautions

These are special safety measures and practices that are used to prevent transmission of the infectious organisms among patients and healthcare workers. Isolation practices are based on the concept of isolating the organism and not the patient. Categories of isolation precautions include:

### I. Standard precautions

- These are a set of infection control practices designed particularly to prevent the transmission of the blood-borne pathogens (HBV, HCV, HIV), in addition to other organisms among patients and healthcare workers.
- The standard precautions assume that every patient is potentially infected; therefore, they should be applied to **all patients all the time** regardless of their infection status.

### II. Transmission-based precautions:

- These are a set of infection control practices applied **in addition** to the standard precautions when a patient has a diagnosed infection. They are designed according to the mode of transmission of this infection.
- They include:
  1. Contact precautions
  2. Droplet precautions
  3. Airborne precautions



Different isolation precautions are presented in table 23:

**Table (23):** Isolation precautions

Type of precaution	Type of patient/infection	Important precaution practices employed
<b>Standard</b>	All patients	<ol style="list-style-type: none"> <li>1. Hand hygiene</li> <li>2. PPE such as gloves, masks, gowns and goggles</li> <li>3. Respiratory hygiene &amp; cough etiquette</li> <li>4. Safe injection practices</li> <li>5. Proper disposal of needles &amp; scalpels</li> <li>6. Proper disposal of the infectious hospital waste</li> <li>7. Cleaning, disinfection &amp; sterilization of equipment</li> </ol>
<b>Contact</b>	<ul style="list-style-type: none"> <li>- Diarrhoea</li> <li>- Wound and skin infection</li> <li>- Infection by multidrug resistant organisms</li> </ul>	<ol style="list-style-type: none"> <li>1. Single room</li> <li>2. Wearing gloves and gowns</li> </ol>
<b>Droplet</b>	<ul style="list-style-type: none"> <li>- Influenza</li> <li>- Mumps</li> <li>- Pertussis</li> <li>- Meningococcal meningitis</li> <li>- Many others</li> </ul>	<ol style="list-style-type: none"> <li>1. Single room</li> <li>2. Wearing standard mask</li> </ol>
<b>Airborne</b>	<ul style="list-style-type: none"> <li>- Tuberculosis</li> <li>- Measles</li> <li>- Chickenpox</li> </ul>	<ol style="list-style-type: none"> <li>1. Single room with negative pressure</li> <li>2. Wearing N95 mask</li> </ol>

**MCQs:**

- 1- The most common organisms causing hospital-acquired infections (HAIs) include all the following **EXCEPT**:
  - a- *Staphylococcus aureus*
  - b- *Klebsiella pneumoniae*
  - c- *Streptococcus pyogenes*
  - d- *Escherichia coli*
  - e- *Pseudomonas aeruginosa*
- 2- Hospitalized patients infected with a multidrug resistant organism, such as MRSA:
  - a- Must be isolated in negative pressure room
  - b- Must be placed in an ordinary single room
  - c- Can be placed in a ward with other patients
  - d- Must be sent home immediately to avoid infecting others
  - e- Can be examined by staff not wearing gloves or gown
- 3- Precautions applied to a patient with pulmonary tuberculosis include:
  - a- Standard precautions only
  - b- Standard precautions and airborne precautions
  - c- Standard precautions and droplet precautions
  - d- Standard precautions and contact precautions
  - e- No precautions are required
- 4- **True or false:**
  - a- Healthcare-associated infections are defined as infections acquired by a patient within 24 hours after admission.
  - b- The wide use of antibiotics in hospitals is a major factor that reduces HAIs.
  - c- Diseases transmitted by droplets include influenza, mumps, rubella, pertussis and tuberculosis.
  - d- Standard precautions are a set of infection control practices that should be applied to all patients regardless of their infection status.
  - e- Airborne precautions include wearing N95 mask.

## MISCELLANEOUS TOPICS

### **ILOs:**

**By the end of this chapter, the student should be able to:**

- Recognize the value of stained smears
- List diseases in which microscopic examination is sufficient for diagnosis
- List diseases in which microscopic examination may be important to start treatment
- Define a carrier
- Classify carriers
- Recognize why carriers are more dangerous than cases
- State the disease conditions in which carriers play an important role
- State schedule of immunization of children in Egypt
- State other childhood vaccines recommended by the WHO
- Define bioterrorism
- List biological agents used in bioterrorism

### **Value of stained smears**

- I. In some diseases, microscopic examination is **sufficient for diagnosis**:
  - Acute gonorrhoea in male patients
  - Bacterial vaginosis
  - Secondary cases of cholera during an epidemic
  - Leprosy
  - Vincent angina
  - Syphilis during the primary stage (chancre)
  - Relapsing fever during the febrile stage
- II. In other diseases, microscopic examination may be important to **start treatment** until the results of culture are available:
  - Meningitis
  - Tuberculosis
  - Diphtheria
- III. The presence of the organism in the stained-smears and its failure to grow in aerobic cultures may point to **anaerobes**.



Carriers

- A carrier is an apparently healthy individual harbouring a pathogenic organism, without having clinical manifestations, and can transmit this organism to others.
- Carriers are more dangerous than cases as a source of infection because they move freely among people without being detected.
- According to the duration of the carriage state, carriers may be: (a) transient carriers e.g. during the incubation period and early convalescence, or (b) chronic carriers e.g. hepatitis-B virus.
- The organism may be discharged from the carrier in an intermittent or a continuous manner.
- Conditions in which carriers play an important role include:
  1. Enteric fever (gall bladder).
  2. Cholera (intestine).
  3. Epidemic cerebrospinal meningitis (nasopharynx).
  4. Diphtheria (throat).
  5. Hepatitis B virus infection (blood).
  6. *S. aureus* carriage (skin and nose)

Immunization of Children in Egypt

Table (24): Schedule of immunization of children in Egypt:

		Birth	2 months	4 months	6 months	15 months	18 months	4-6 years
BCG		+						
DPT			+	+	+		+	+
OPV			+	+	+		+	+
HBV			+	+	+			
MMR						+		+

BCG = Bacille Calmette & Guerin, DPT = Diphtheria-Pertussis-Tetanus, OPV = Oral Polio Vaccine, HBV = Hepatitis B Vaccine, MMR = Measles-Mumps-Rubella.

- Other childhood vaccines recommended by the WHO include:
  - Hib vaccine (*Haemophilus influenzae* type b vaccine)
  - PCV vaccine (Pneumococcal conjugate vaccine)
  - Rota virus vaccine
  - Meningococcal vaccine
  - Influenza vaccine
  - HAV vaccine (Hepatitis A virus vaccine)
  - Varicella vaccine

## Biological Warfare and Bioterrorism

- Bioterrorism is defined as the intentional use of biological agents that cause disease or death in people, animals or plants. These agents may be bacteria, viruses or toxins. They may be in a naturally occurring or a genetically modified form to increase their virulence or resistance to drugs or vaccines.
- They are classified into categories, based on the ease of dissemination and their ability to cause morbidity and mortality.
- Agents in the highest risk category are:
  - Anthrax
  - Smallpox
  - Botulism
  - Viral haemorrhagic fever (e.g., Ebola virus)
  - Plague

### MCQs:

- 1- The disease conditions in which human carriers play an important role include all of the following EXCEPT:
  - a- Enteric fever
  - b- Cholera
  - c- Diphtheria
  - d- Plague
  - e- Hepatitis B virus infection
- 2- Agents in the highest risk category as causes for bioterrorism include the following EXCEPT:
  - a- Anthrax
  - b- Smallpox
  - c- Botulism
  - d- Diphtheria
  - e- Viral haemorrhagic fever
- 3- Diseases in which microscopic examination is sufficient for diagnosis include the following EXCEPT:
  - a- Leprosy
  - b- Tuberculosis
  - c- Bacterial vaginosis
  - d- Vincent angina
  - e- Acute male gonorrhoea

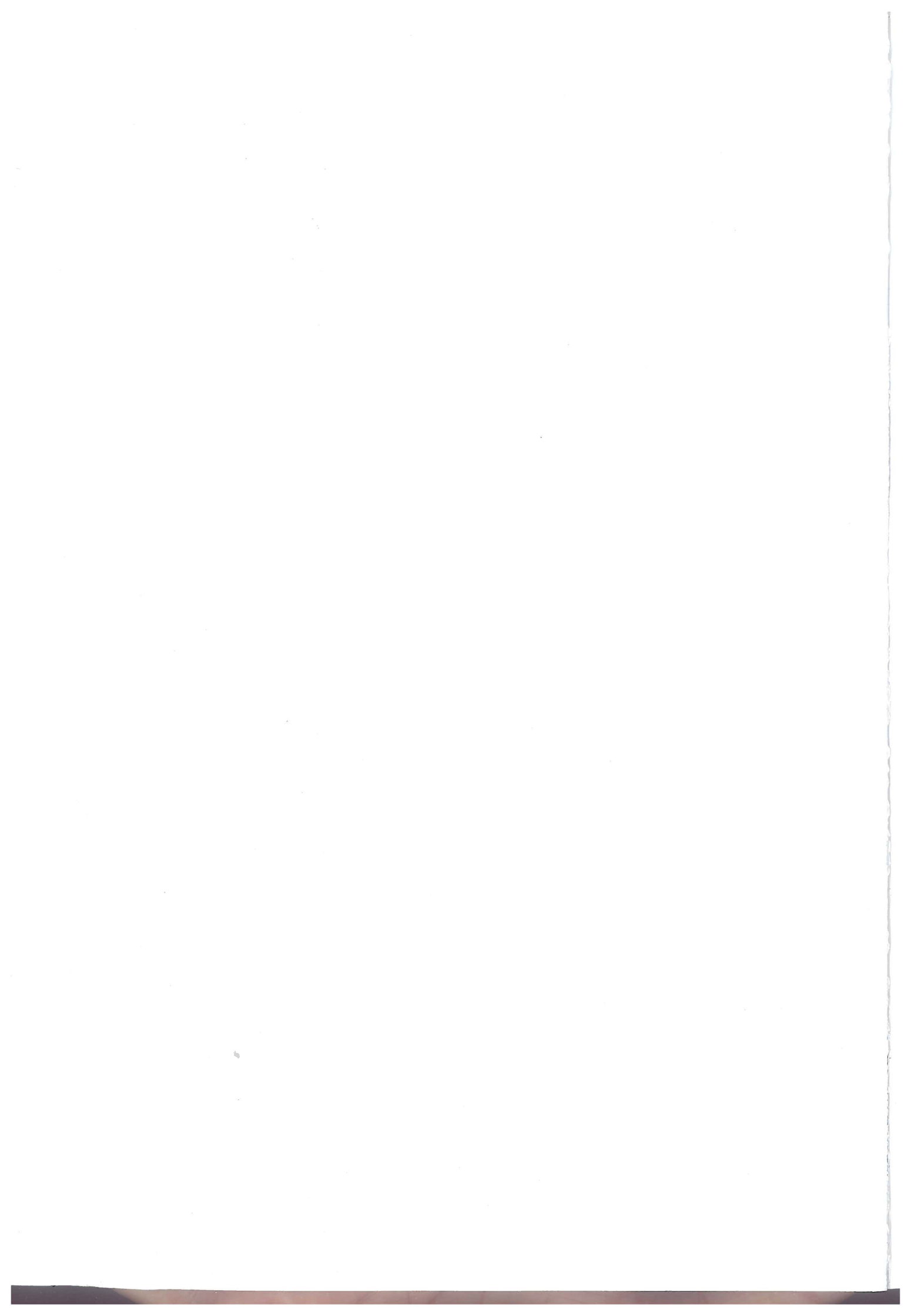
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Chapter 11:	1 d	2 b	3 d								
Chapter 12:	1 a	2: a F	b F	c T	d T	e F					
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Chapter 18:	1 a	2: a F	b T	c T							
Chapter 19:	1 c	2 d									
Chapter 20:	1 d	2 a									
Chapter 21:	1 d	2 e	3 d								
Chapter 22:	1 a	2 e									
Chapter 23:	1 c	2 d	3: a F	b T	c i F	ii T	iii T	iv T	v F	vi F	vii T
Chapter 24:	1 b	2 a	3 b	4 d	5 b	6 d					
Chapter 25:	1 b	2 d	3 c	4 d	5 b						
Chapter 26:	1 c	2 b	3 b	4: a F	b F	c F	d T	e T			
Chapter 27:	1 d	2 d	3 b								





# ESSENTIAL MEDICAL MICROBIOLOGY and IMMUNOLOGY

Practical  
Microbiology



**ESSENTIAL  
MEDICAL MICROBIOLOGY  
AND IMMUNOLOGY**

**Practical Microbiology**

**Fifth Edition**

**By**

***Staff Members of  
Medical Microbiology and Immunology Department***

**Faculty of Medicine-Cairo University  
2018-2019**





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## Chapter 1

# INFECTION CONTROL MEASURES IN THE MICROBIOLOGY LABORATORY

Measures taken to reduce the risk of infection within the laboratory environment comply with the standard precautions of infection prevention and control, and include general safety precautions, strict use of personal protective equipment and hand hygiene.

H-H

S-P

PPE

## Safety in the Laboratory

Safety in the microbiology laboratory is important in the prevention of:

- 1- Infection that might be caused by the microorganisms being handled.
- 2- Hazards that might be caused by the harmful chemicals used in the laboratory.
- 3- Injuries that might be caused by glassware, open flames, and sharp objects if used improperly.

النسب

### General Guidelines:

- Upon entering the laboratory, all personal belongings should be kept away from workstations.
- Hands should be washed with soap at the start of the laboratory session before performing any procedure and before leaving the laboratory at the end of the session.
- Safety protection equipment should be used (Fig. 1, 2)
  - Disposable latex gloves are essential to prevent hand contamination.
  - Lab coats are required at all times.
  - Safety goggles are recommended when splashing is expected.
  - Sandals should not be worn because of the danger of accidental cuts from broken glass or the possibility of chemical spills.

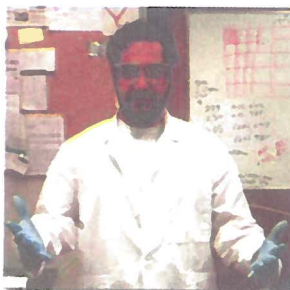


Fig. (1): PPE in the lab.  
(coat, gloves, goggles)



Fig. (2): Footwear in the lab.



- Smoking in the lab is essentially prohibited (Fig. 3).
- No eating or drinking in the lab. This means no gum, candy, holding a pencil in the mouth, nail-biting..... etc.
- Long hair must be tied back or covered to minimize fire hazard or contamination of experiments.



**Fig. (3): NO eating / NO drinking / NO smoking**

- Everything should be handled as if it is contaminated by pathogenic organisms.
- Flame and flammable solutions should be kept far apart.
- All sharps (e.g., needles, razors, pins, toothpicks) should be discarded in a sharps container (Fig. 4).



**Fig. (4): Sharp box**

- All cultures should be handled as being potentially pathogenic. These instructions should be followed:
  - Liquid cultures must always be kept in a test tube rack.
  - Broth cultures must never be pipetted by mouth.
  - Spilled cultures should be covered with paper towels and then saturated with a disinfectant solution. After 15 minutes, the towels should be removed and disposed of in a red bag (for dangerous/infectious waste) (Fig. 5).
- All accidents no matter how minor, should be reported to the lab instructor/supervisor.



**Fig. (5): Dealing with blood spills**

# Personal Protective Equipment (PPE)

PPE are specialized clothing or equipment worn by health care personnel (HCP) for protection of:

- HCP clothing and skin from contamination by patients' blood and body fluids.
- Patients from acquiring microorganisms from HCP.

## Types of PPE

### A) Gloves (Fig. 6):

There are different types of gloves:

#### 1- Sterile gloves:

They are mostly used for surgery and invasive procedures. They are disposable and individually wrapped.

#### 2- Non-sterile gloves:

They are mostly used to protect skin against exposure to blood and body fluids e.g. when removing soiled dressings and handling specimens.

Both types of gloves should be discarded after one use followed by hand hygiene.

#### 3- Utility or heavy-duty household gloves:

They are used for handling contaminated items and waste. They can be reused after decontamination.



Fig. (6): Types of gloves

### B) Gowns and Aprons:

Gowns or aprons should be used during procedures that are likely to generate splashes of blood or body fluids, e.g. surgical operations (Fig. 7).

### C) Eye Goggles:

They should be used for protection against splashes of blood or body fluid generated during certain procedures, e.g. surgical operations (Fig. 8).

#### D) Overhead:

Disposable caps should be worn to contain hair during surgical procedures. They should be well-fitting and sealed (Fig. 9).

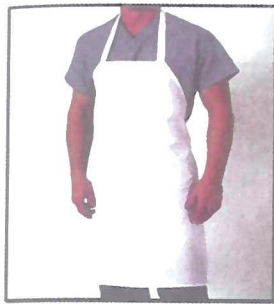


Fig. (7) Apron



Gown



Fig. (8) Eye goggles



Fig. (9): overhead

#### E) Masks:

They should cover nose, mouth and chin (including beard). There are different types of masks:

##### 1- Standard masks (Fig. 10):

They are used when there is risk of exposure to droplets that might contain infectious agents.

##### 2- N95 masks (Fig. 11):

They are used when there is risk of exposure to airborne infectious agents such as *M. tuberculosis*. These high efficiency masks are designed to capture high percentages (>95%) of particles that are less than 1 micron in size.



Fig. (10): Standard mask



Fig. (11): N95 mask

#### F) Footwear:

Overshoes are **not recommended**, as it is an ideal way of transferring microorganisms from floor and shoes to hands. Instead, closed footwear is needed in special areas such as the operating theatre (Fig. 12).



Fig. (12): Closed foot wear  
in operating theatre



Over-shoes  
NOT recommended



# Hand Hygiene

*"The 10 most common ways of spreading infections are the 10 fingers."*

Hand hygiene is the **simplest** and **most effective** measure to prevent transmission of hospital-acquired infections. It is part of the 'Standard Precautions'.



## Microbial Flora of the Skin

Microbial flora of the skin can be divided into 2 categories:

### a) Resident microorganisms:

These are organisms that are **permanently** found in deeper layers of the skin (permanent residents). If disturbed, they reestablish themselves. They are not removed by mechanical friction during hand-washing with soap and water, but can usually be partially killed or inhibited by antimicrobial agents.

Organisms of the resident flora are usually not associated with hospital-acquired infections.

### b) Transient microorganisms:

These are organisms that **temporarily** colonize the superficial layers of the skin. They are acquired through interactions with patients and with the environment. They are easily mechanically removed by hand-washing with soap and water.

Organisms of the transient flora are the organisms most frequently associated with hospital-acquired infections.

## When is Hand Hygiene done? (Fig. 13)

- 1- Before touching a patient.
- 2- Before aseptic techniques e.g. handling a medication.
- 3- After touching a patient.
- 4- After touching blood, body fluids or items contaminated with them.
- 5- After touching patients' surrounding.
- 6- After removing gloves.

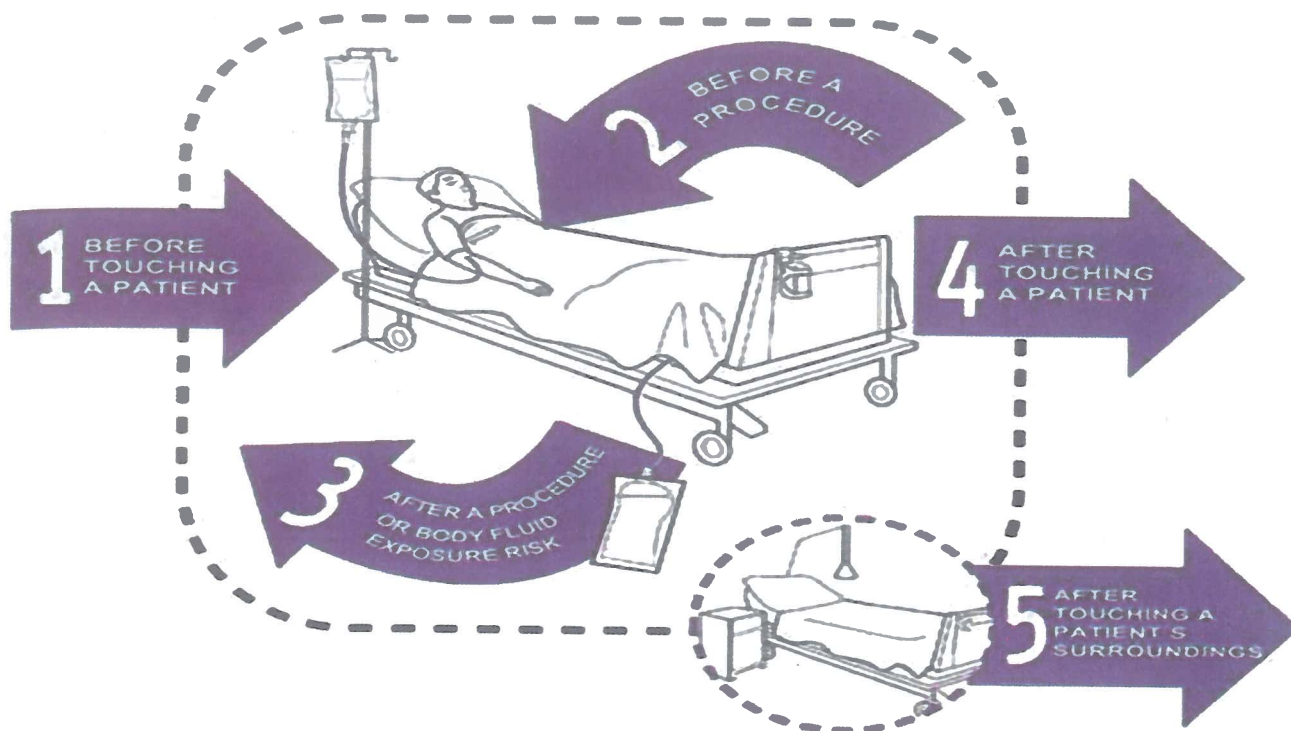


Fig. (13): When to wash hands?

### Types of Hand Hygiene:

#### I) Routine hand wash:

This is done by applying soap and water. It removes dirt, organic material and most of the transient flora.

#### II) Antiseptic hand hygiene (alcohol hand-rub):

This is done by applying 70%-90% alcohol. It kills transient flora and reduces resident microorganisms.

It is not effective if hands are visibly soiled.

#### III) Surgical hand scrub:

It is a procedure done before surgery using e.g. alcohol or iodophores (betadine) for at least 5 minutes. Antiseptic agent should be applied up to the elbows.

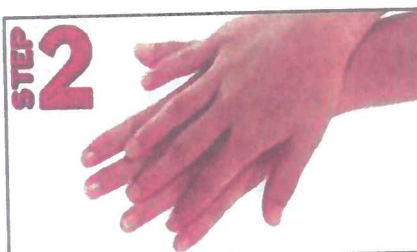
### Steps of routine hand wash (Fig. 14):

- 1- Remove jewellery and watches.
- 2- Turn on tap.
- 3- Wet hands.
- 4- Apply enough soap.
- 5- Rub hands as shown in figure 3C (Part II).
- 6- Rinse thoroughly.
- 7- Dry hands with clean paper towel.
- 8- Turn off tap with paper towel or elbows (without re-contaminating hands).

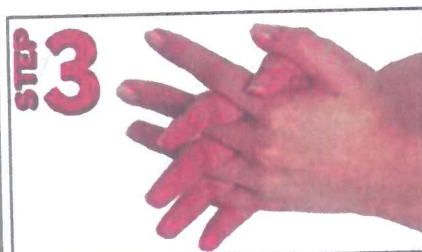




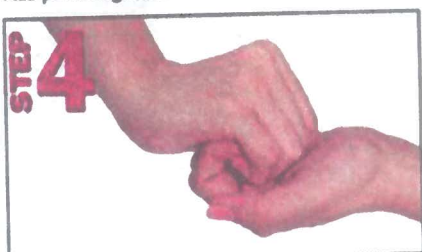
STEP 1  
Rub palms together.



STEP 2  
Rub the back of both hands.



STEP 3  
Interlace fingers and rub hands together.



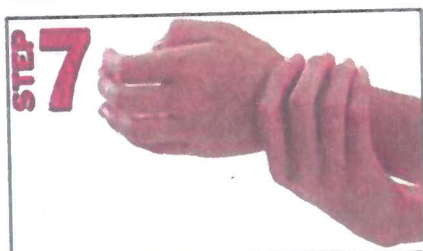
STEP 4  
Interlock fingers and rub the back of fingers of both hands



STEP 5  
Rub thumb in a rotating manner followed by the area between index finger and thumb for both hands.



STEP 6  
Rub fingertips on palm for both hands.



STEP 7  
Rub both wrists in a rotating manner. Rinse and dry thoroughly.



Fig. (14) Steps for hand wash

Remember: *Clean Hands are Healing Hands!*



## Chapter 2

# LABORATORY DIAGNOSIS OF INFECTIOUS DISEASES

## (A) Bacterial Infections

The laboratory diagnosis of infectious diseases involves 2 main diagnostic methods:

- I) **Direct method:** which depends on the detection of microorganisms, their structural components or their products in specimens collected from patients (e.g. urine, blood, sputum or CSF).
- II) **Indirect methods:**
  1. Serologic method: which depends on detection of antibodies against the microorganism in the patient's serum.
  2. Other methods: e.g. skin tests and QuantiFERON-TB test.

### I) DIRECT METHOD

#### 1) Specimen Collection:

Direct method requires the collection of a "good quality" clinical specimen from the patient. This is dependant on:

- a) Collecting specimens before the start of antibiotics.
- b) Choosing the appropriate specimen (representing the infection site).
- c) Using sterile containers and avoiding contaminating the specimen.
- d) Transporting the specimen properly to the lab. as early as possible.

#### 2) Microscopic Examination:

Specimens collected are usually examined microscopically before their further processing. Microscopic examination of stained or unstained (wet) preparations may be done using different types of microscopes.

#### 3) Microbial Detection:

Following microscopic examination, specimens are further processed for direct diagnosis by culture or non-culture techniques.

- a) **Culture technique:** which includes procedures involving
  - The isolation of the organism in pure culture (by inoculating the specimen onto appropriate artificial culture media), followed by
  - Identification of the isolate using different approaches, for example:
    - Microscopic examination.
    - Biochemical reactions.
    - Serologic identification of the organism by reaction with specific antibody.
    - DNA probes.

Which of these approaches is used and in what sequence depends upon the type of specimen and the suspected organism.

**N.B.:** After the organism is grown in pure culture, its sensitivity to various antibiotics is determined.

**b) Non-culture technique:** which includes procedures involving

- The identification of a **specific microbial antigen** such as a structural component (e.g. cell wall antigen or capsular polysaccharide) or a microbial product (e.g. an exotoxin) **directly in the specimen** by reacting with specific antibody.
- The identification of a **specific gene sequence** (i.e. nucleic acid of the organism) by the application of different molecular methods (e.g. PCR or DNA probe).

As these non-culture techniques do not depend on growth and multiplication of the organism, they yield more rapid results (minutes or hours); however, antimicrobial susceptibility cannot be determined (although the presence of resistance genes can be detected by molecular methods).

Non-culture techniques are mainly applied if:

- a) A rapid diagnosis is needed
- b) The microorganism cannot be cultured on artificial media
- c) The patient has received antimicrobial therapy
- d) The pathogen is a slowly growing organism

## II) INDIRECT METHODS

**1- Serologic diagnosis** of infectious diseases involves the use of known microbial antigens to detect **antibodies against the microorganism** in the patient's serum.

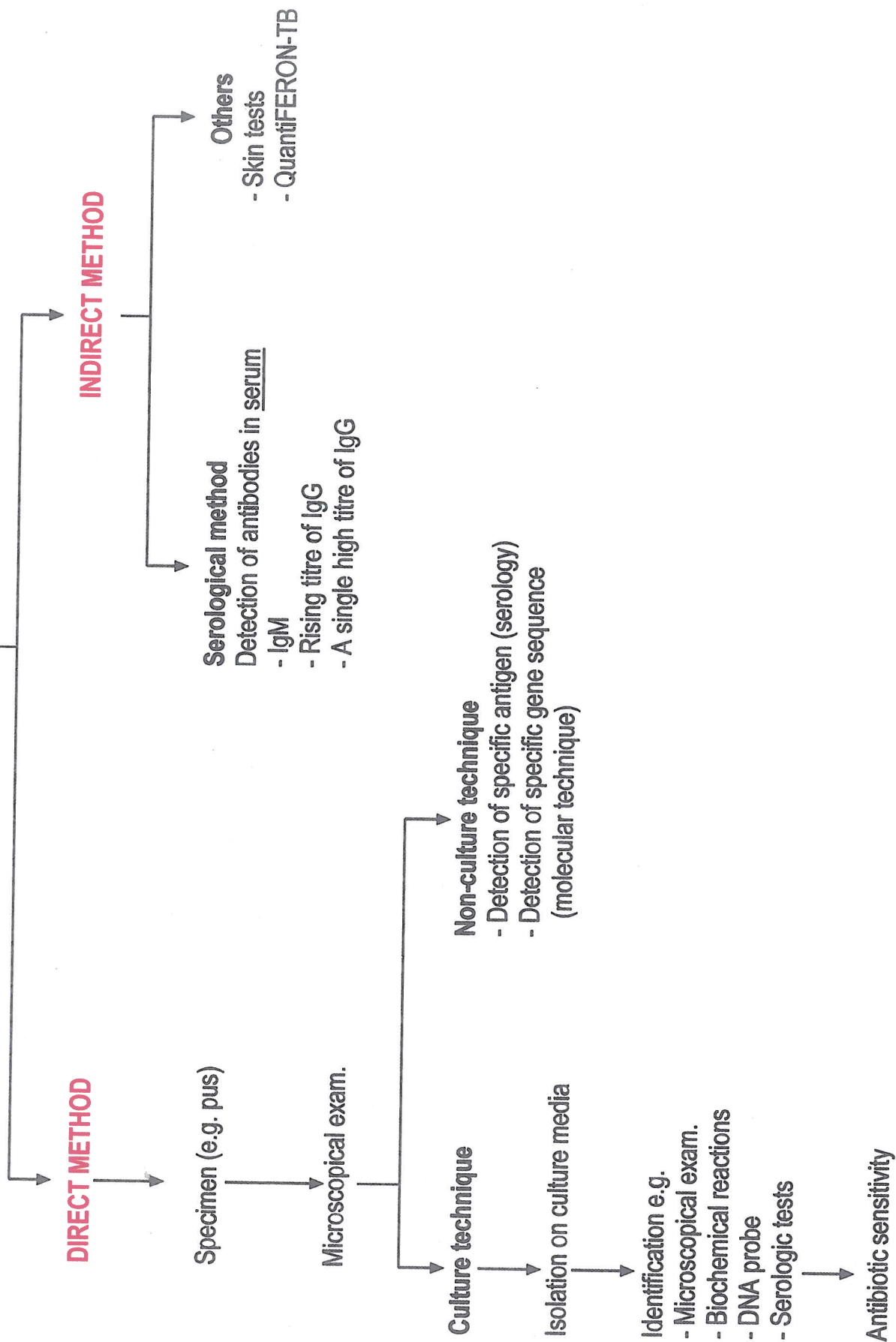
Detection of one of the following indicates current (active) infection:

- a) Specific IgM antibodies.
- b) Rising titre of specific IgG antibodies (4-fold or greater rise).
- c) A single high titre of IgG antibodies in certain diseases.

**2- Other methods:**

- a) **Skin tests** based on cell-mediated hypersensitivity.
- b) **Quantiferon-TB test** based on estimation of IFN- $\gamma$  release by sensitized T-lymphocytes.

## Diagnosis of bacterial infections





# Microscopic Examination

Microorganisms are too small (measured in microns) to be seen by the naked eye. For this reason, they are visualized by the use of microscopes.

Microscopical examination allows us to study the morphology of bacteria which is the first step in identification. Morphological study includes: size (0.2-14  $\mu\text{m}$ ), shape (e.g. cocci, bacilli, vibrios), arrangement (e.g. clusters, chains, pairs), motility and staining characteristics.

Different types of microscopes have been developed.

## Microscopes

### A- Light microscope: (Fig. 15)

- **Magnifications:**

- a- Eye piece x 10.

- Objective lenses:

- Low power x 10 (final magnification: 100).

- High power x 40 (final magnification: 400).

- Oil immersion lens x 100 (final magnification: 1000).

- **Uses:**

- 1- Studying the morphology of bacteria: In this case, stained preparations are examined using the oil immersion lens with a final magnification of 1000 (one micron will be magnified to one millimeter).

- 2- Studying the motility of bacteria: In this case, fresh unstained preparations are examined (hanging drop preparation) using the low and high power of the microscope.



Fig. (15): Light microscope

### B- Dark-field (dark-ground) microscope: (Fig. 16)

In this type of microscopes the light is directed obliquely (by a special condenser) so that it cannot pass through the lenses unless reflected by bacteria (e.g. spirochaetes) present in the examined specimen.

**Uses:**

Studying unstained preparations of spirochaetes which are too delicate to be seen by the light microscope. They will appear as motile illuminated spiral bodies against a dark background.

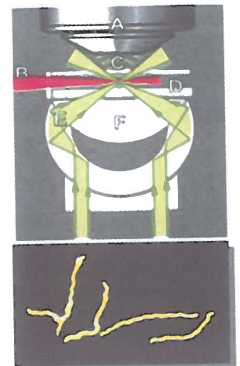
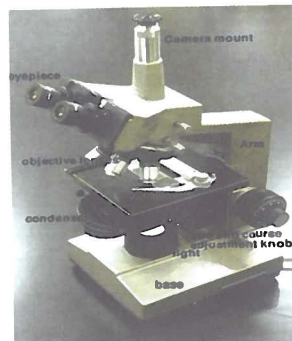


Fig. (16): Dark field microscope

### C- Fluorescent (ultraviolet) microscope: (Fig. 17)

When using this type of microscopes, organisms are stained with dyes which give fluorescence on exposure to ultraviolet light.

#### Uses:

- 1- Studying the morphology of bacteria by staining them with a fluorescent dye, e.g. auramine-O which is used to stain tubercle bacilli.
- 2- Performing immunofluorescent serologic assays.

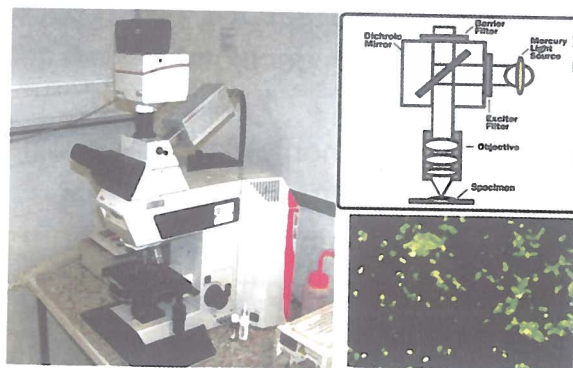


Fig. (17): Fluorescent microscope

### D- Electron microscope: (Fig. 18)

- In this type of microscopes, illumination is obtained by an electron beam (which has very short wave length). The electron beam passes through an evacuated column and is focused on the object by means of an electromagnetic condenser.
- Electron microscope has a magnifying power of 100,000 or more.

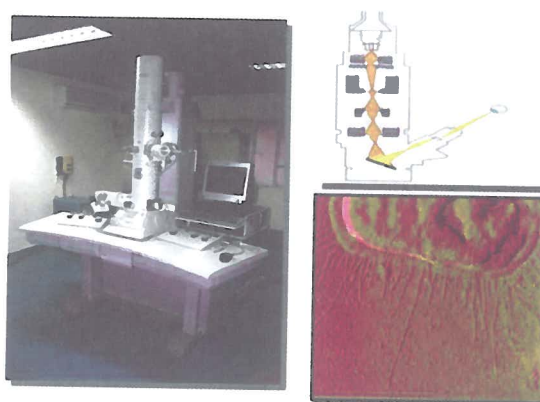


Fig. (18): Electron microscope

#### Uses:

- 1- Studying the ultra-structure of bacteria and tissue cells.
- 2- Studying the structure of viruses and rickettsia.

## Staining of Bacteria

There are many techniques for staining bacteria. The most commonly used are simple staining techniques and differential staining techniques. Special staining may be indicated, e.g. Fontana stain for spirochaetes, Giemsa stain for rickettsia, and auramine-O stain for *M. tuberculosis*.

**A- Simple stains:** one dye, e.g. methylene blue, is used. All bacteria stained will take the colour of the dye.

**B- Differential stains:** two dyes, separated by a decolourizing agent are used:

- The first dye (primary stain) is applied. All bacteria will take the colour of the dye.

- A decolourizing agent is used. Some bacteria are decolourized and others will retain the dye.
- When the second dye (counterstain) is applied, the bacterial cells which have been decolourized will take the colour of the counterstain.

The most commonly used differential stains are **Gram stain** and **Ziehl-Neelsen stain** (Fig. 19, 20).

**N.B.:** Before staining, a smear from the sample is spread on a clean glass slide, and fixed by passing it over the flame.

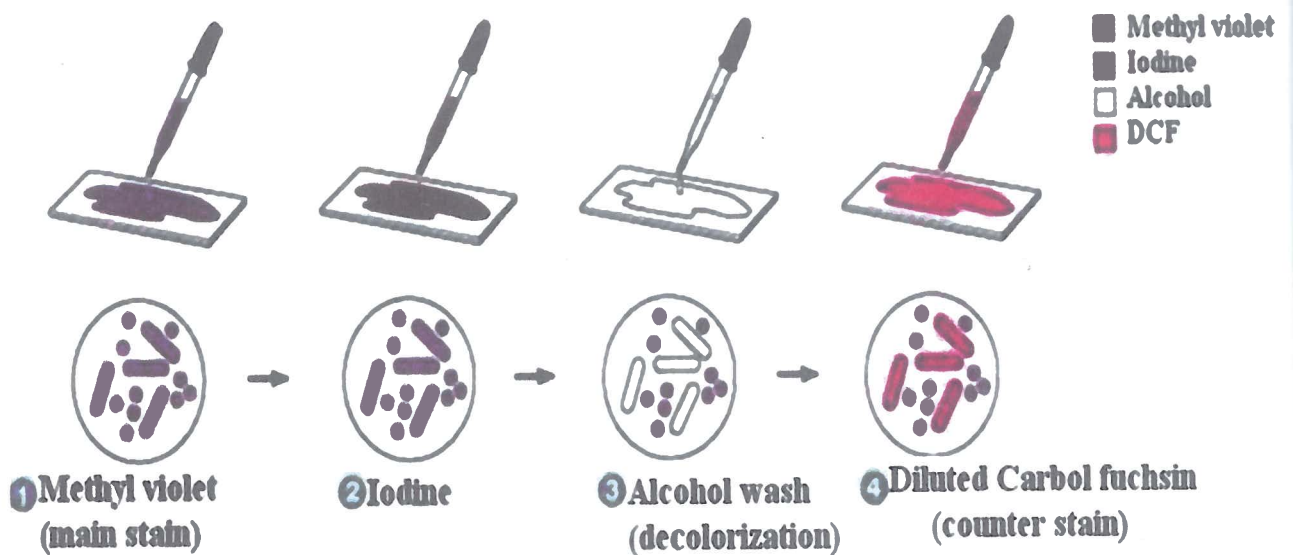


Fig. (19): Gram staining technique

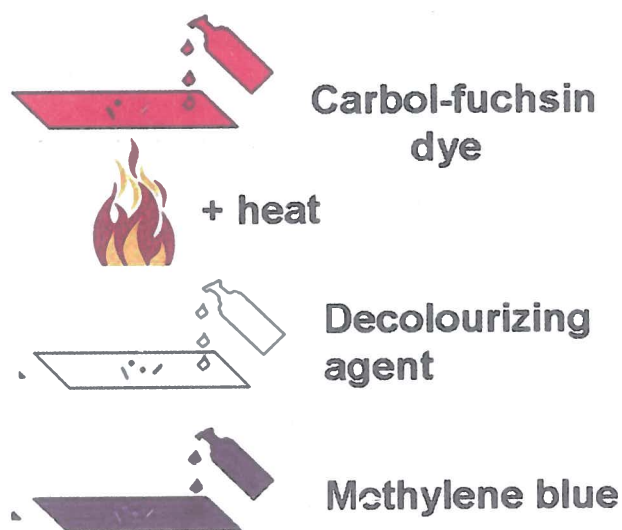
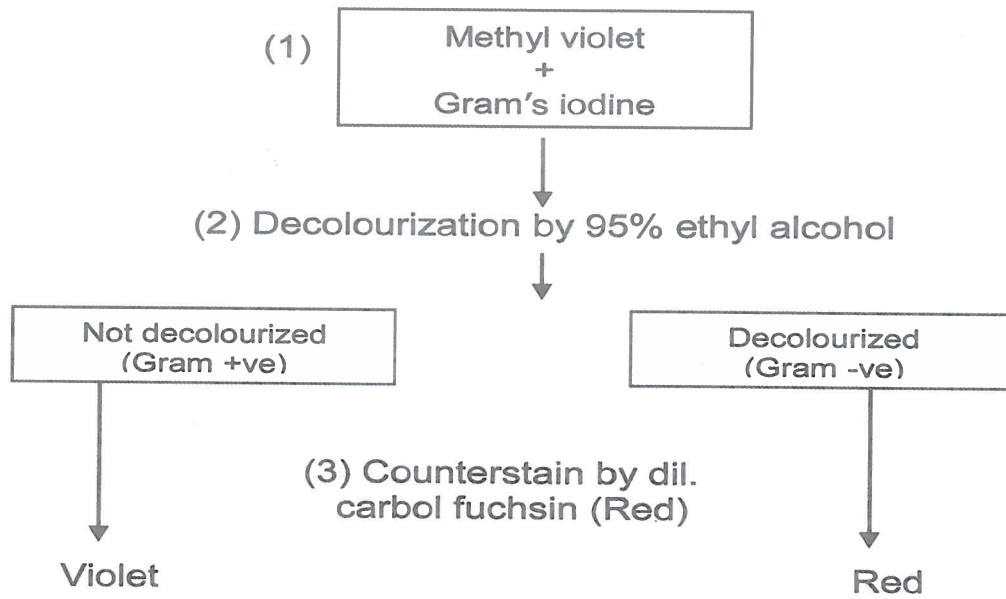


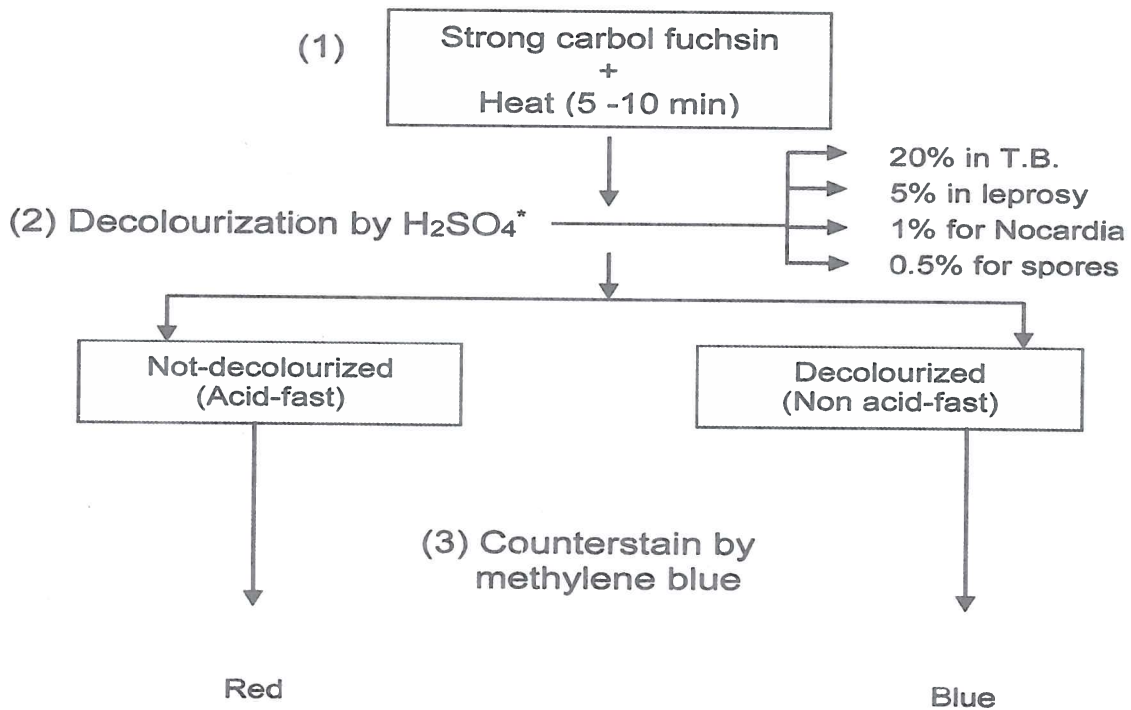
Fig. (20): Ziehl Neelsen stain



## Gram's Stain



## Ziehl-Neelsen Stain



\* N.B.: Decolourization can be done by 3% HCl in alcohol.

## Value of stained smears

- I. In some disease conditions, microscopical examination is sufficient for diagnosis:
  - Acute gonorrhoea in male patients.
  - Bacterial vaginosis.
  - Secondary cases of cholera during an epidemic.
  - Leprosy.
  - Vincent angina.
  - Syphilis during the primary stage (chancre).
  - Relapsing fever during the febrile stage.
- II. In other disease conditions, microscopical examination may be important to start treatment until the results of culture are available:
  - Meningitis.
  - Tuberculosis.
  - Diphtheria.
- III. The presence of the organism in the stained-smears and its failure to grow in aerobic cultures may point to anaerobes.

## Culture of Bacteria

The purpose of using cultural techniques is to demonstrate the presence of organisms causing the disease and to test their sensitivity to antibiotics (if indicated).

The clinical specimen is inoculated on carefully selected culture media to provide the optimal conditions for growth and multiplication of bacteria (essential nutrients, gas requirements, optimal pH ..... etc).

According to its physical state, culture media may be liquid or solid. Solid media are preferable because:

- 1- Solid media allow isolation of the organism present in a mixture in **pure culture** for further studies. In liquid media, organisms grow in mixture and the medium becomes turbid.
- 2- Solid media help in **identification** of the organism (different organisms have different colonial morphology and different effects on various media).

### The plating out technique: (Fig. 21)

- Using a sterile loop, the clinical specimen is smeared over area A to give a main inoculum.
- The loop is resterilized by flaming, cooled, and 3 parallel lines (B) are drawn from the main inoculum on to the surface of the medium.

- This process is repeated (lines C and D) with sterilizing and cooling the loop between each sequence.

This technique ensures that bacteria from the infective material are ultimately deposited singly and at sufficient distance from each other so that the whole progeny of each single bacterium accumulates locally during growth to form a **colony**, seen by the naked eye. Each colony (being the progeny of a single bacterium) is presumed to be a pure culture.

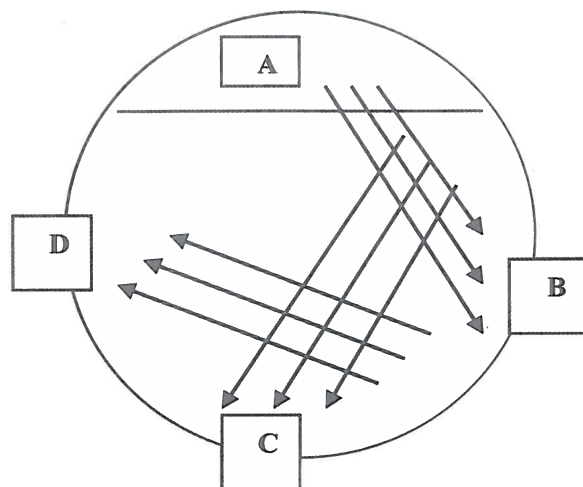


Fig. (21): Plating out technique

## Culture Media

There are various kinds of culture media which can be grouped according to the chemical composition into the following categories:

**A- Simple (basal) media:** They provide the basic requirements for the growth of most bacteria. Examples include:

- 1- **Peptone water:** A simple fluid medium containing essentially peptones and amino acids (Fig. 22)
- 2- **Nutrient broth:** A simple fluid medium formed of meat extract (Fig. 22).
- 3- **Nutrient agar:** It is nutrient broth solidified by addition of 2% agar-agar. Agar-agar has no nutritive value (Fig. 23).

Peptone water, nutrient broth and nutrient agar are sterilized by autoclaving.

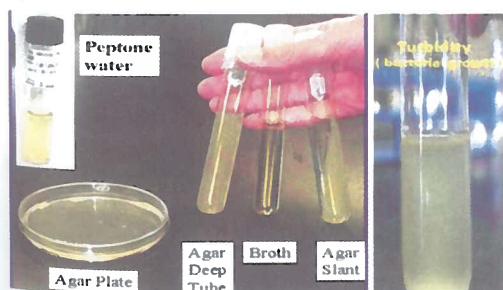


Fig. (22): Simple media



Fig. (23): Nutrient agar



**B- Enriched media:** They are prepared to meet the nutritional requirements of fastidious bacteria. This is done by the addition of blood, serum, yeast extract, egg..... etc, to a basal medium. Examples include:

- 1- **Blood agar** is prepared by adding 5-10% blood (sheep, horse or human), collected under aseptic precautions, to sterile nutrient agar melted and cooled to 50°C (Fig. 24). In addition to being an enriched medium, it is an indicator medium showing the haemolytic properties of bacteria e.g. streptococci, may cause complete ( $\beta$ ), partial ( $\alpha$ ) or no haemolysis (Fig. 25 ).



Fig. (24): Blood agar medium

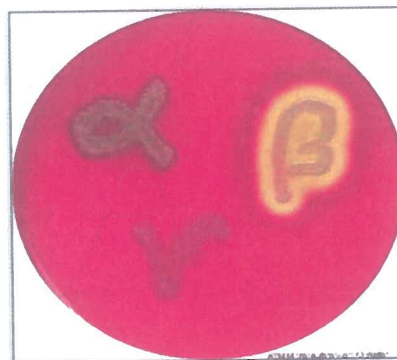


Fig. (25):Types of haemolysis on blood agar

- 2- **Chocolate agar (heated blood agar)** is prepared as blood agar medium then reheated to 98°C. The heat ruptures the red cells and changes haemoglobin to haematin which is chocolate brown in colour. This medium is suitable for cultivation of *Haemophilus* and *Neisseria* (Fig. 26).

- 3- **Loeffler's serum medium** consists of serum (e.g. horse serum) and glucose broth. The medium is rendered solid by heating at 80°C for 1 hour resulting in coagulation of serum proteins. The medium is suitable for growth of *C. diphtheria* (Fig. 27).

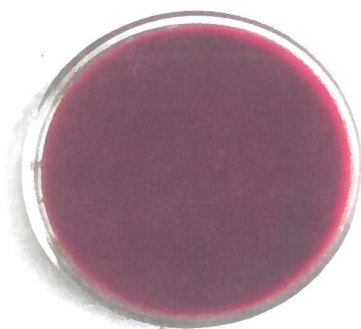


Fig. (26): Chocolate agar for *Neisseria* and *Haemophilus*

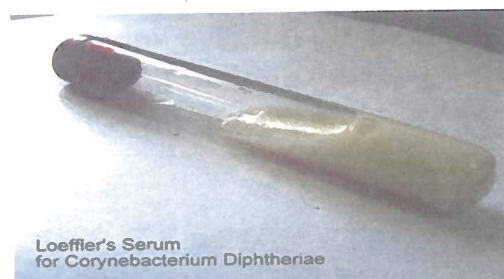


Fig. (27): Loeffler's serum for *C. diphtheriae*

**C- Selective media:** These media contain substances that inhibit the growth of some organisms but allow the growth of a certain organism, thus, facilitating its isolation from a mixture. These inhibitory substances may be dyes, chemicals or antibiotics. Examples include:

- 1- **Lowenstein Jensen medium** contains beaten eggs, and is rendered selective for growth of *M. tuberculosis* by addition of malachite green dye. The medium is solidified by heating at 80°C, resulting in coagulation of egg proteins (Fig. 28).
- 2- **Blood tellurite** is a blood agar rendered selective for isolation of *C. diphtheriae* by addition of potassium tellurite (Fig. 29).
- 3- **Modified Thayer-Martin medium (MTM)** is a chocolate agar rendered selective by adding certain antibiotics. It is used for isolation of pathogenic *Neisseria* (Fig. 30).

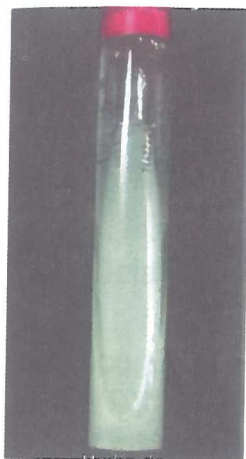


Fig. (28): L-J medium for *M. tuberculosis*

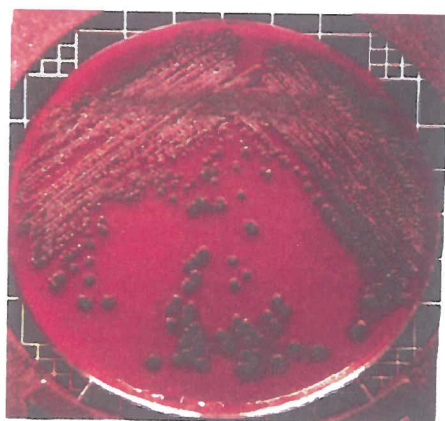


Fig. (29): *C. diphtheriae* on blood tellurite

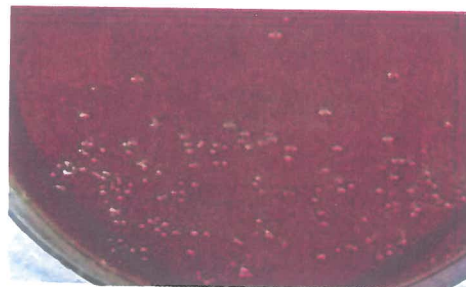


Fig. (30): Gonococci on Modified Thayer- Martin

**D- Enrichment media:** These are liquid media with selective properties. Examples include:

- 1- **Selenite broth** is probably the most commonly used enrichment medium for isolation of *Salmonella* and *Shigella* from stools (Fig. 31).
- 2- **Tetrathionate broth** may also be used to isolate *Salmonella* from stools (Fig. 31).
- 3- **Alkaline peptone water (pH 8.6)** is a useful medium for isolation of *V. cholerae* from faeces and contaminated materials (Fig. 32).



Fig. (31): Selenite broth      Tetrathionate broth

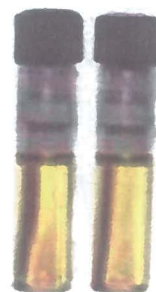


Fig. (32) Alkaline peptone water



**E- Differential (indicator) media:** These media contain some substances that are changed visibly as a result of metabolic activity of a particular organism. For example:

**Triple sugar iron (TSI) agar:** This medium is composed of (Fig. 33):

- 3 sugars: 0.1% glucose, 1% lactose and 1% sucrose as test sugars.
- Phenol red (pH indicator): for detection of acid production due to sugar fermentation.
- Ferrous sulphate for detection of H<sub>2</sub>S production.
- Beef extract and yeast extract for nutrition.
- 0.5% agar for solidification (soft agar that cracks easily upon gas production).

The medium is poured in test tubes in the form of a slant and a deep butt.

Sterile uninoculated TSI medium is transparent red in colour. The tubes are inoculated with the isolated organism and incubated at 37°C for 24 hours.

- Bacteria with no fermentative activity give red slant and red butt e.g. *Pseudomonas aeruginosa*.
- Bacteria that ferment glucose only (non lactose fermenter) give red slant and yellow butt, e.g. *Salmonella* and *Shigella*.
- Bacteria that ferment glucose, lactose and/or sucrose give yellow slant and yellow butt, e.g. *E.coli* and *Klebsiella*.
- Some bacteria produce gas which causes the agar to crack, e.g. *E. coli* and *Klebsiella*.
- Bacteria that produce H<sub>2</sub>S give blackening of the butt, e.g. *Proteus*.

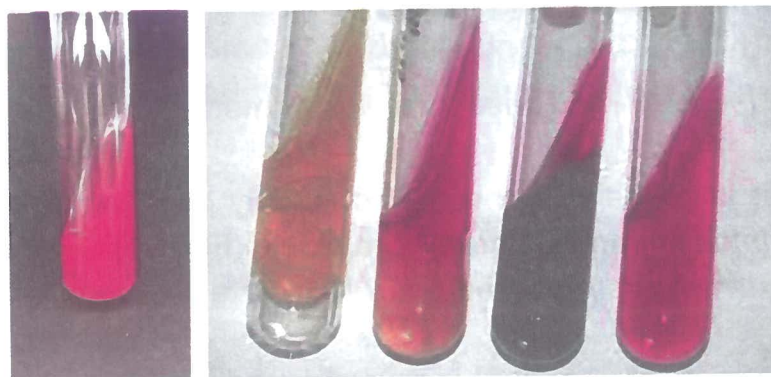


Fig. (33): Triple sugar iron (TSI)

Table (1): Triple sugar iron (TSI) agar reactions

Organism	Slant	Butt	Gas	H <sub>2</sub> S
<i>E. coli</i> & <i>Klebsiella</i>	Acid (yellow)	Acid (yellow)	+ (cracks)	-
<i>V. cholerae</i>	Acid (yellow)	Acid (yellow)	-	-
<i>Shigella</i>	Alkaline (red)	Acid (yellow)	-	-
<i>Salmonella</i> & <i>Proteus</i>	Alkaline (red)	Acid (yellow)	±	+ (black)
<i>Pseudomonas</i>	Alkaline (red)	Alkaline (red)	-	-



**F- Selective and indicator media:** These media contain selective substances which allow the growth of a certain range of organisms; they also contain an indicator which permits the differentiation between these organisms. Examples include:

- 1- **MacConkey's agar** contains bile salts that render the medium selective for enteric bacteria. It contains lactose (a test sugar) and neutral red (pH indicator). The medium is used to distinguish lactose fermenting organisms (e.g. *E. coli* which gives pink colonies) from lactose nonfermenting organisms (e.g. *Salmonella* which gives pale colonies) (Fig.34, 35).



Fig. (34): MacConkey's medium



Fig. (35): Lactose fermenter and lactose non-fermenter on MacConkey's medium

- 2- **Deoxycholate Citrate Agar (DCA)** contains bile salts, citrate and other substances to inhibit normal intestinal flora. It also contains lactose and neutral red. It is superior to MacConkey's medium in isolation of *Salmonella* and *Shigella*.

3- **TCBS:**

- The medium contains thiosulphate, citrate and bile salts (selective substances), sucrose (test sugar) and bromothymol blue (pH indicator). The medium has alkaline pH which increases its selectivity (Fig. 36).
- The medium is used for isolation of *V. cholerae* which produces yellow colonies due to fermentation of sucrose and production of acid that changes the colour of the indicator into yellow (Fig. 37).



Fig. (36): T.C.B.S. medium

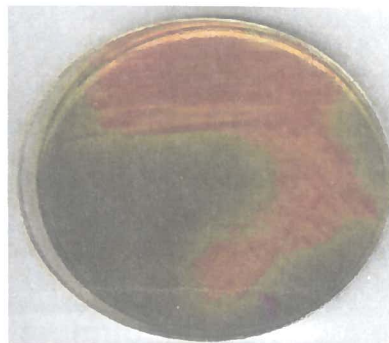


Fig. (37): *Vibrio cholerae* on T.C.B.S.

## Anaerobic Culture

Obligate anaerobes will not grow in culture unless oxygen is absent. This can be achieved either by exclusion of oxygen or by addition of reducing substances to culture media.

The most commonly used anaerobic liquid media are:

- 1- **Thioglycollate broth** in which sodium thioglycollate is added as a reducing agent (Fig. 38).
- 2- **Cooked meat broth** in which cooked minced meat is added to broth. The meat contains haematin, glutathione and ascorbic acid which act as reducing agents (Fig. 39).

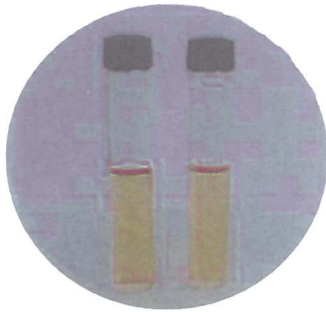


Fig. (38): Thioglycollate broth for anaerobic cultivation

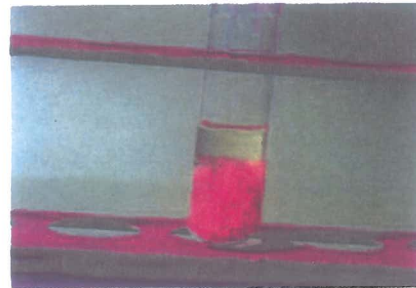


Fig. (39): Cooked meat medium for anaerobic Cultivation

### Gaspak system: (Fig. 40)

- For culture of anaerobes on solid media, blood agar plates are recommended. The inoculated plates are placed in an anaerobic jar (Gaspak system).
- In this system, hydrogen and CO<sub>2</sub> are generated inside the jar from commercially available Gaspak envelopes.
  - The released hydrogen combines with the oxygen inside the jar providing anaerobic atmosphere.
  - The released CO<sub>2</sub> enhances the growth of many clinically important anaerobes.

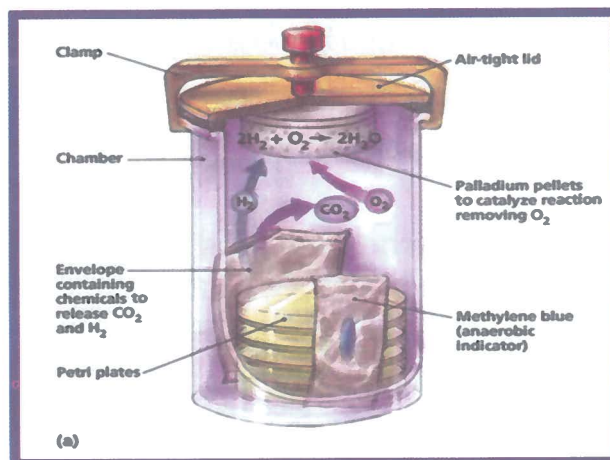


Fig. (40) Gas Pak System for anaerobic cultivation (Anaerobic jar)



## Blood Culture

Blood culture is required whenever bacteraemia (or septicaemia) is suspected. In most cases of bacteraemia, the organisms are not numerous in the blood and, so, direct plating of a drop of the patient's blood will usually give a negative result. Therefore, for diagnosis of such infections the **blood culture technique** is applied as follows (Fig. 41):

- Large volume of blood (5-10 ml) is withdrawn under strict aseptic conditions.
- The sample is added to blood culture bottles containing 100 ml broth and incubated at 37°C.
- Subcultures are done on suitable solid media every 48 hours and continued for up to 10 days (sometimes longer) before the sample is discarded as negative.
- Isolated organisms are identified in a systematic way.

To increase the chance for isolating a pathogen:

- 1- It is necessary to obtain large volume (5-10 ml) of blood.
- 2- Three blood cultures are done. These should be taken from different sites and collected at different times due to the intermittent presence of the organism in the blood.
- 3- Large volume of broth is used which has the following advantages:
  - a. It reduces the concentration of natural antimicrobial constituents in the blood to a sub-inhibitory level.
  - b. It allows the growth and multiplication of the small number of organisms present in the blood sample.
- 4- In patients under antibiotic therapy, neutralization of antibiotics in blood samples or reducing their effect may be done by different methods.

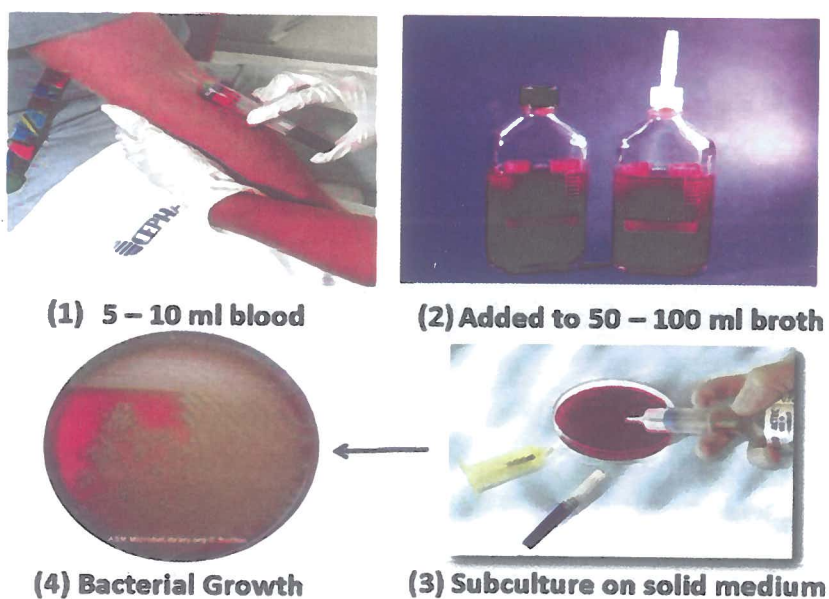


Fig. (41) : Blood culture technique



## Common infectious diseases diagnosed by blood culture:

- Enteric fever
- Brucellosis
- Endocarditis
- Meningitis
- Puerperal sepsis

In some organisms, the colony morphology (shape, size, pigment production, consistency ... etc) and its effect on the culture media, together with the microscopical morphology of the organism may be sufficient for diagnosis, e.g.

a- Gram negative, motile bacilli showing greenish blue exopigment on nutrient agar is *Pseudomonas aeruginosa*.

b- Gram negative, motile bacilli showing swarming growth on nutrient agar is *Proteus*.

In many other conditions, it may be necessary to proceed for further diagnostic procedures.

## Biochemical Reactions

Bacteria vary in their metabolic and enzymatic activities. This can be used in identification of different genera and species of bacteria. For this reason, one or more of the following biochemical reactions may be done. Pure cultures of bacteria are necessary for performance of biochemical tests.

### I. Reactions depending on sugar fermentation:

1- **Sugar fermentation tests:** Bacterial species can be identified by the pattern of their fermentative activities on different sugars. They produce either acid only or acid and gas.

2- **Methyl red (M.R.) test:** Some bacteria (e.g. *E. coli*) when grown on glucose phosphate peptone can produce large amount of acid (from glucose fermentation). This reduces the pH of the medium below 4 and the colour of the added M.R. indicator becomes red (M.R. +ve). A negative test gives a yellow colour (Fig. 42).

3- **Voges-Proskauer (V.P.) test:** Some bacteria (e.g. *Klebsiella pneumoniae*) when grown on glucose phosphate peptone produce acetyl methyl carbinol from glucose fermentation. The production of acetyl methyl carbinol can be detected by addition of concentrated KOH which gives a pink colour (V.P. +ve). A negative test gives a yellow colour (Fig. 43).

any fermentation to any sugar  $\xrightarrow{\text{acid \& gas}}$  acid & gas

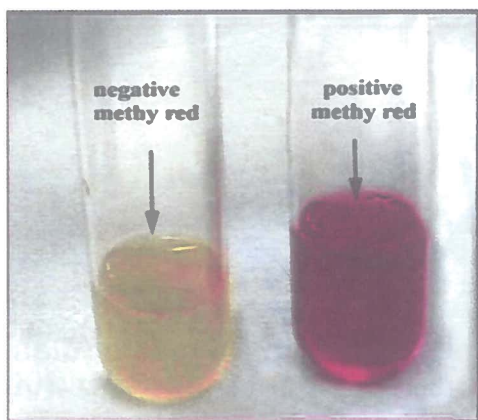


Fig. (42): MR

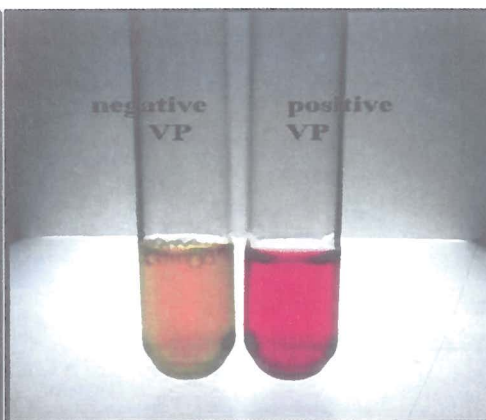


Fig. (43): VP

## II. Reactions depending on amino acid metabolism:

- 1- **Indole test:** Some bacteria (e.g. *E. coli* and *Vibrio cholerae*) can act on tryptophane (an amino acid present in peptone water) with production of free indole. This can be demonstrated by addition of Kovac's reagent which gives a red ring (indole +ve). A negative test gives a yellow ring (Fig. 44).
- 2- **H<sub>2</sub>S production:** Some bacteria (e.g. *Salmonella Typhi*) can decompose the sulphur-containing amino acids present in the medium and form H<sub>2</sub>S (see TSI medium) (Fig. 45).

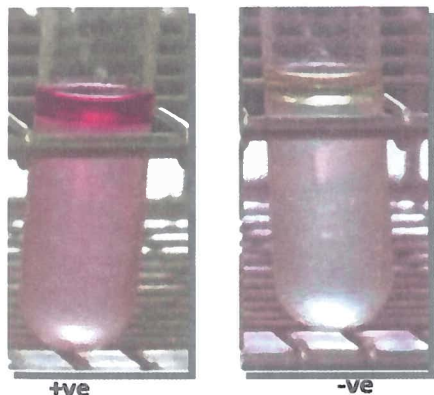


Fig. (44) : Indole test

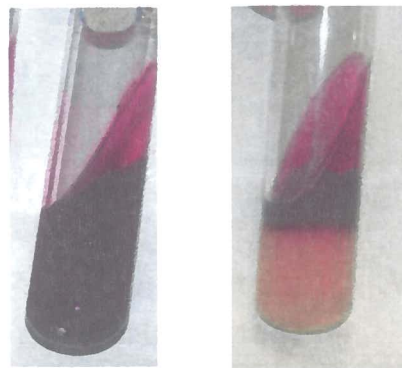


Fig. (45) : H<sub>2</sub>S production

## III. Reactions depending on enzyme production:

### 1- Catalase test (Fig. 46):



Some bacteria (e.g. *S. aureus*) produce catalase enzyme. If a colony of such bacteria is added to a drop of hydrogen peroxide, O<sub>2</sub> bubbles will be released immediately (catalase +ve).

### 2- Coagulase test (Fig. 47):

*S. aureus* produces coagulase enzyme which changes fibrinogen into fibrin.

- This can be detected by mixing the organism with plasma in a test tube.
- Production of coagulase results in clot formation within few hours .

Pointed Coagulase  $\rightarrow$  Fleming factor

Free Coagulase  $\rightarrow$  Bakterien factor

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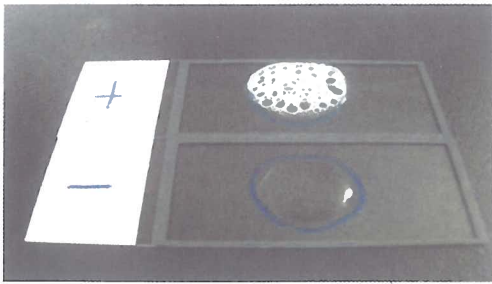


Fig. (46): Catalase test

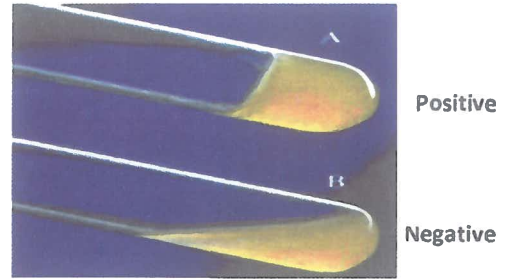


Fig. (47): Coagulase test

- 3- **Urease test:** Some organisms (e.g. *Proteus*) produce urease enzyme. If they are grown on urea-containing media, ammonia is released and the phenol red indicator turns red (urease +ve) (Fig. 48).
- 4- **Oxidase test:** Some bacteria (e.g. *Neisseria* and *Pseudomonas*) produce oxidase enzyme. If cultures of such bacteria are treated with oxidase reagent (tetramethyl-p-phenylene diamine hydrochloride), a deep purple colour is produced within few seconds (oxidase +ve) (Fig. 49).



Fig. (48): Urease test

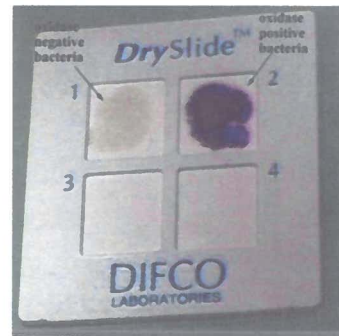


Fig. (49): Oxidase test

#### IV. Other reactions:

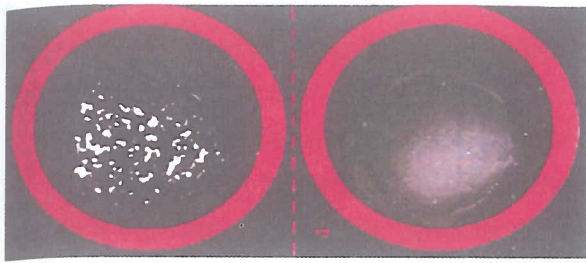
##### 1- Test for clumping factor:

- The clumping factor (adhesin) is a cell surface protein which mediates binding of *S. aureus* to fibrinogen.
- It can be detected by mixing a drop of bacterial suspension with a drop of plasma on a slide.
- Presence of clumping factor leads to aggregation of the organism within few seconds (Fig. 50).

- 2- **Citrate utilization test:** Some bacteria (e.g. *Klebsiella pneumoniae*) are able to use citrate as their only source of carbon. The test is performed by inoculation of the organism in Simmon's citrate medium, where the original green colour of the medium turns into bright blue colour (citrate +ve) (Fig. 51).



لا يكتسب شكل Citrate يتحول من لونه لالزرق



*S. aureus*

*S. epidermidis*

Fig. (50): Clumping factor test

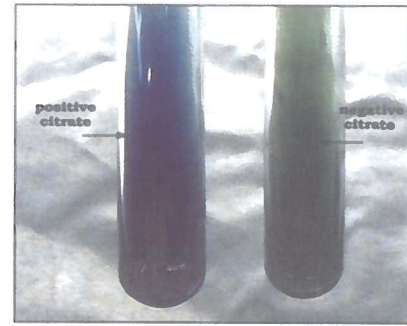


Fig. (51): Citrate test

## Commercial API Kit Systems (Analytical Profile Index)

Packaged biochemical identification systems are available from commercial sources. It consists of a plastic strip containing a series of small cupules, each containing dehydrated media and reagents. A suspension of the test organism is dropped in the hole at the top of the cupule and incubated at 37°C for 24-48 hours. The biochemical profiles are determined and interpreted according to the charts given with the kits (Fig. 52).

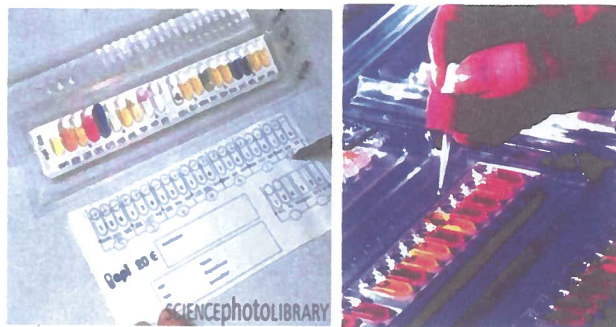


Fig. (52): Analytical Profile Index (API)

## Serological Identification of Bacteria

Bacteria have different antigens which correspond to different structures of the cell, e.g. somatic or "O" antigen, flagellar or "H" antigen and capsular antigens.

An unknown bacterium can be identified by demonstrating its reaction with a known specific antiserum (antibodies) using different antigen antibody reactions, e.g. agglutination and precipitation (Fig. 53).

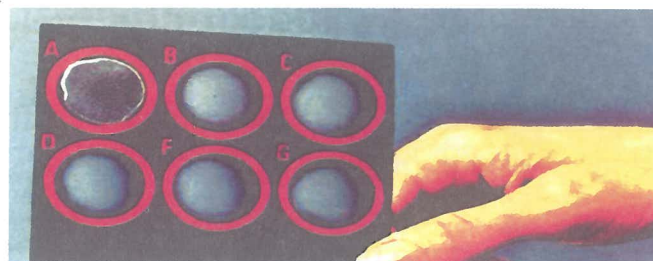


Fig. (53): Slide agglutination test for serological identification of an organism

## Bacterial Typing

- A given species of bacteria can be classified into several types. Typing or 'fingerprinting' of individual strains of an organism is a major factor in epidemiological investigations, such as tracing the source of infection in an outbreak of post-operative wound sepsis or an outbreak of food poisoning.
- The bacterial strain isolated from the patients should be of the same type as that isolated from the suspected source(s).
- Different typing methods are available for different organisms. Examples include: typing by colony morphology, bacteriophage-typing, biotyping, pyocin-typing, serotyping, plasmid analysis, ribotyping, chromosomal analysis and others.

### Bacteriophage typing of *S. aureus* is done as follows:

- The strain to be tested is inoculated on the surface of a suitable solid medium and left to dry.
- From each phage, a drop is placed in a marked square area on the surface of the plate and incubated at 30°C.
- After 24 hours incubation, a homogeneous growth of bacteria will appear on the surface of the plate except for areas of lysis denoting susceptibility to this phage. These areas will determine the phage type of the strain (Fig. 54).

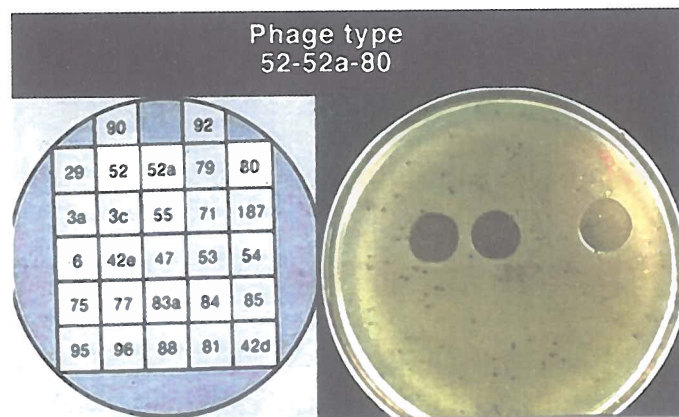


Fig. (54): Phage typing

## In Vitro Antibiotic Susceptibility Tests

Testing the sensitivity or resistance of the isolated pathogenic bacteria to antibiotics is indicated for selection of the drug which is most suitable for treatment of the patient.

*In vitro* sensitivity testing can be done by one of 3 methods:



### 1. Disc diffusion method (Fig. 55):

- It is a semiquantitative, rapid and easy test for routine use in the laboratory.
- Filter paper discs, each containing a standard amount of an antibiotic, are placed on the surface of a suitable solid medium that has been seeded with the test organism.
- After incubation, the diameter of the clear zone of inhibition surrounding the disc is taken as a measure of the inhibitory power of the drug against the organism.

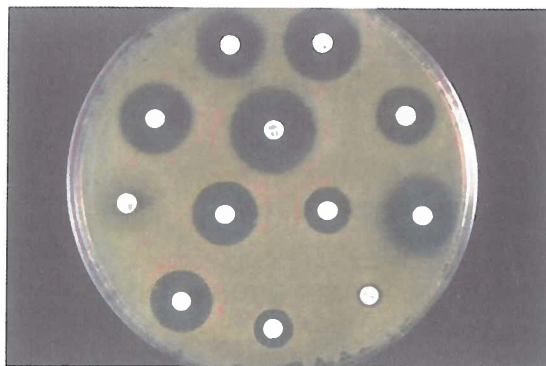


Fig. (55) Antibiotic sensitivity test (Disc diffusion method)

### 2. Dilution method (Fig. 56):

- It is a quantitative method used to determine the minimal inhibitory concentration (MIC) of an antibiotic for a particular organism.
- Serial dilutions of the drug are incorporated into a suitable liquid medium. The media are subsequently inoculated with the test organism and incubated.
- The highest dilution of the antibiotic which inhibits the growth of the test bacteria is referred to as MIC.

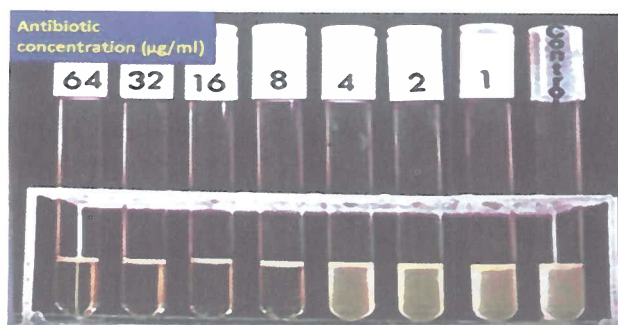


Fig. (56): Tube dilution method (for MIC determination)

### 3. E-test (Fig.57):

- This is another quantitative technique for measuring MIC.
- For each antibiotic, a test strip is used which contains a predefined, continuous, increasing gradient of antibiotic concentrations.
- The strip is applied to the surface of a suitable medium inoculated with the test organism.
- After overnight incubation, a tear drop-shaped (elliptical) inhibition zone is seen. The zone edge intersects the graded test strip at the MIC of the antibiotic.

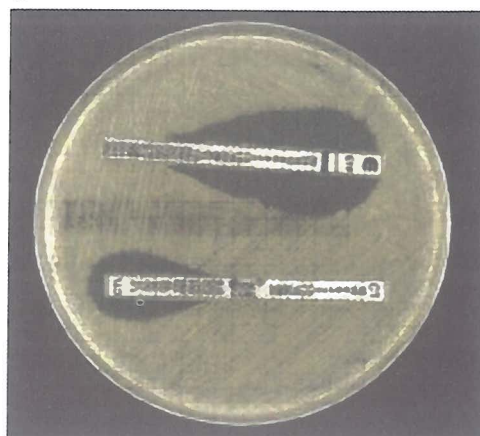


Fig. (57): E-test (for MIC determination)



## (B) Viral Infections

Th  
a-  
b-  
c-

The laboratory diagnosis of viral infections is done on the same lines as that of bacterial infections. It involves 2 main diagnostic methods:

**A- Direct methods:** which depend either on the detection of viruses and/or their components in the patient's specimens, or on isolation of viruses.

**B- Indirect methods:** which depend mainly on the detection of antibodies against the suspected virus in the patient's serum, or on skin tests.

The different techniques used in diagnosis of viral infections can be summarized as follows:

### A- Direct Methods:

**I. Direct detection of viruses and/or their components:** The following procedures are used:

a-  
Pie  
gro  
Vir

- 1- **Light microscopy:** The light microscope can be used in examination of large viruses as poxviruses, or giant cells in herpes infection, or inclusion bodies, e.g. Negri bodies in nerve cells in rabies.
- 2- **Electron microscopy (EM):** This method is used when large number of viruses is present in the sample. It also gives an idea about the size and shape of viruses.
- 3- **Immunoelectron microscopy (IEM):** This is done by the addition of antibodies specific to the virus suspected to be present in the clinical sample. This will lead to aggregation of the unknown virus particles. It is used to detect viruses that cannot be cultured, e.g. rota virus and hepatitis A virus in stools.
- 4- **Fluorescent microscopy:** Using direct immunofluorescent antibody technique (IF), e.g. diagnosis of rabies in brain smears.
- 5- **Immunoassays:** For detection of the virus antigens (e.g. hepatitis B antigens) in blood by the use of either ELISA or RIA.
- 6- **Nucleic acid hybridization:** It is a highly sensitive and specific method. Specific labeled probes are added to clinical samples. These probes will hybridize with the complementary nucleic acid of the virus in the specimen.
- 7- **Polymerase chain reaction (PCR):** It is a method in which amplification of a short sequence of a target nucleic acid of the virus allows it to be easily detected by different methods, e.g. probes.

### II. Isolation of viruses:

- The specimen is taken from sites or lesions suspected to contain the virus.
- Antibiotics are added to prevent bacterial contamination.

There are three main methods for cultivation of viruses in the laboratory (Fig. 58):

- a- Cell culture (tissue culture)
- b- Embryonated eggs
- c- Laboratory animals

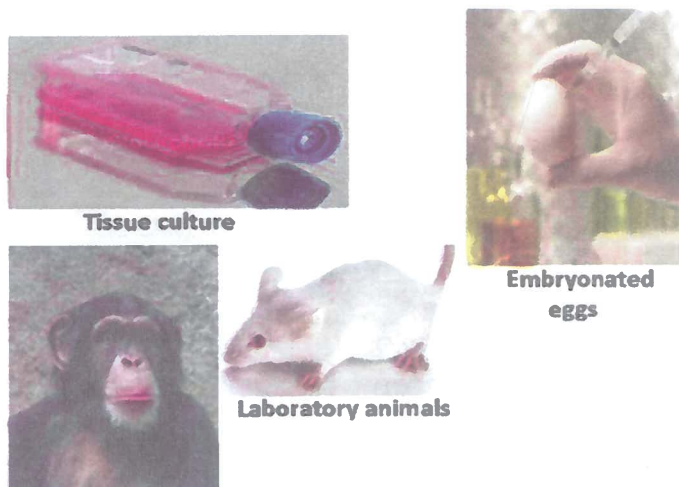


Fig. (58): Methods of viral cultivation

### a- Cell culture (tissue culture) (Fig. 59)

Pieces of tissues are treated with trypsin to get separate cells. These cells are then grown in bottles, tubes or plates to form an attached monolayer culture (Fig. 60). Viruses are inoculated on this sheet of cells and incubated at 37°C for 24-48 hours.

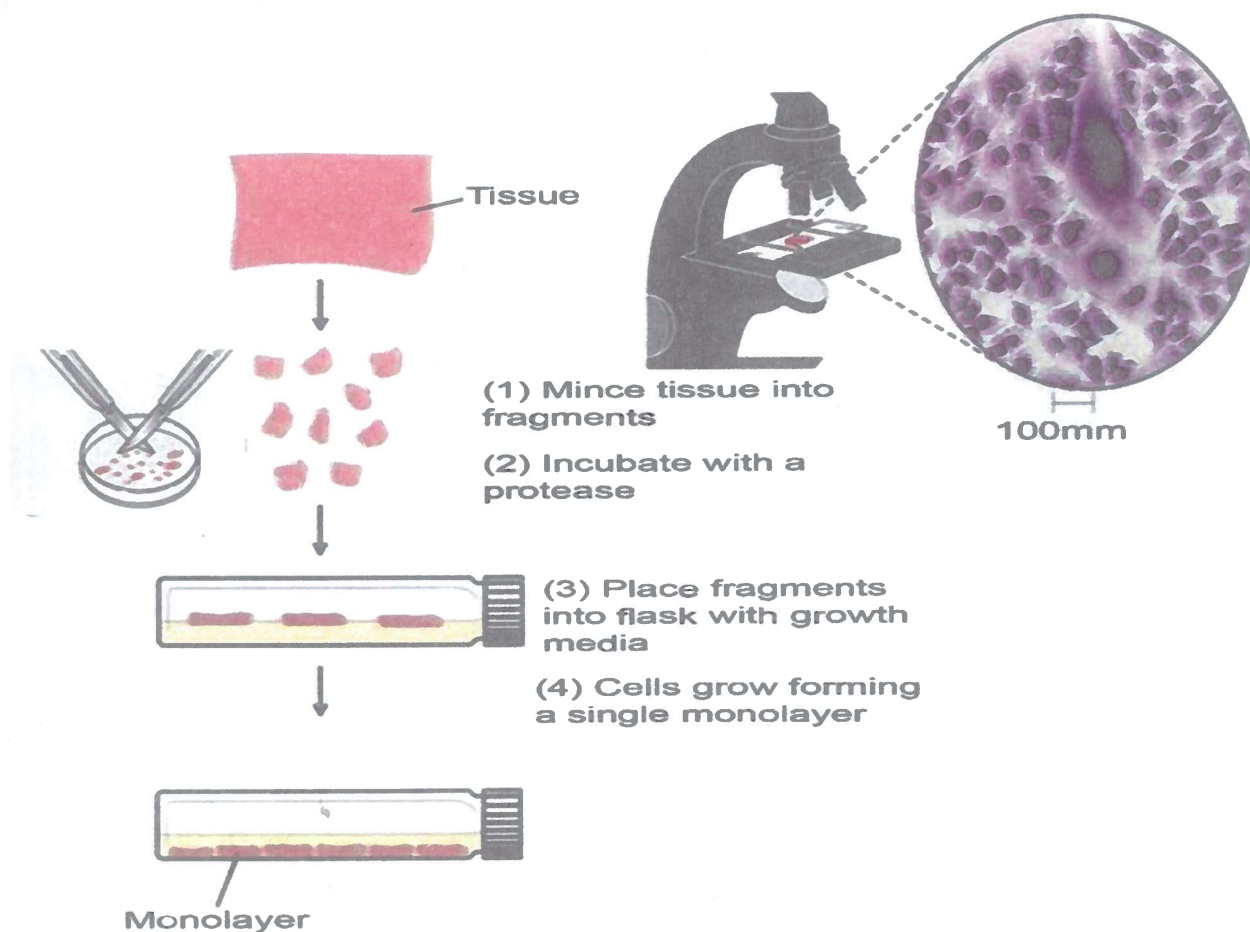
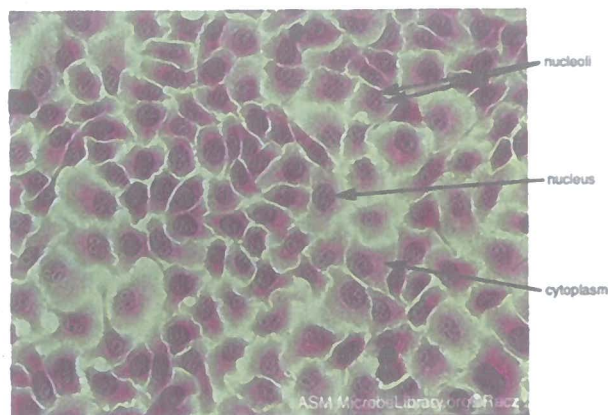


Fig. (59): Cell (tissue) culture





**Fig. (60): Cell line (monolayer)**

3

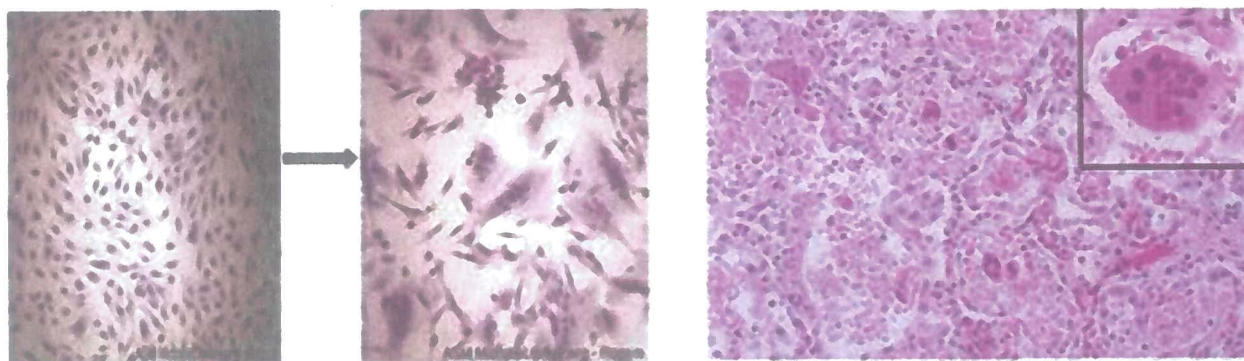
### Types of cell culture:

- 1- **Primary cell lines:** Prepared from organ fragments, e.g. monkey kidney. They can be subcultured for a limited number (5-10 passages).
- 2- **Diploid cell lines:** Prepared from human embryo tissue, e.g. lung. They can be subcultured for 50-100 passages.
- 3- **Continuous cell lines:** Prepared from tumour cells with unlimited number of subcultures or passages, e.g. HeLa cells from carcinoma of cervix.

**Detection of virus replication in cell culture:** Virus growth can be detected by:

- 1- **Cytopathic effect (CPE):** CPE are the morphological changes in the infected cells seen microscopically. This occurs with most viruses and includes:
  - a. Cell death or lysis (Fig. 61)
  - b. Syncytial formation (giant cells) (Fig. 62): due to fusion of membranes of adjacent cells to form multinucleated giant cells, e.g. in case of respiratory syncytial virus (RSV), measles and mumps viruses.

4-



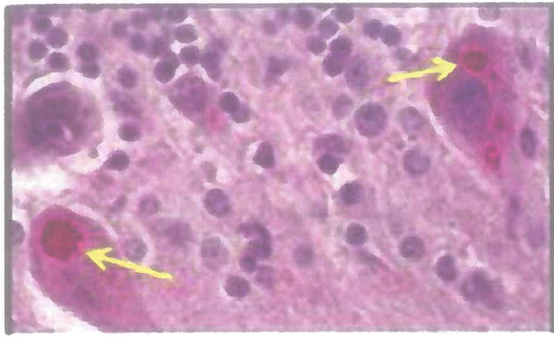
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**Fig. (61): Cell lysis or death (CPE)**

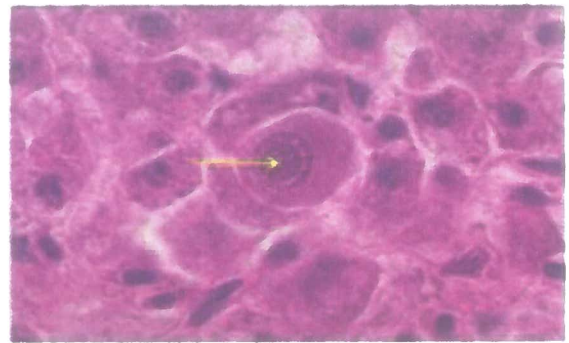
**Fig. (62): Syncytial formation (CPE)**

- 2- **Inclusion bodies:** These are either the site of virus assembly or degenerative changes in the cell. They may be:
  - a. Intracytoplasmic (Fig. 63): e.g. rabies virus (Negri bodies).
  - b. Intranuclear (Fig. 64): e.g. herpes viruses.
  - c. Both: e.g. measles virus.



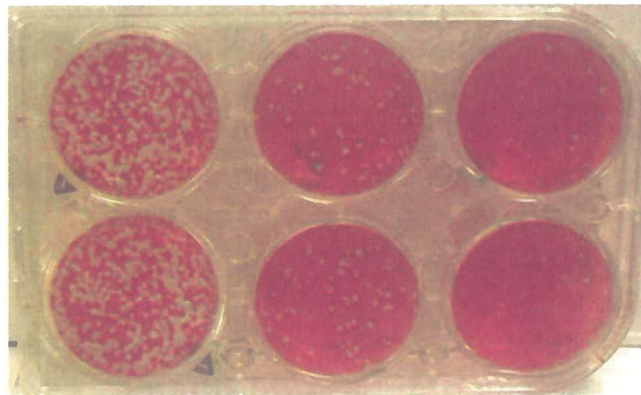


**Fig. (63): Inclusion bodies (intracytoplasmic)**



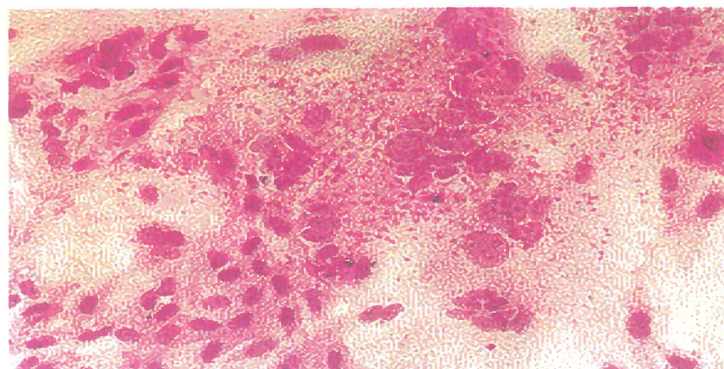
**Fig. (64): Inclusion bodies (intranuclear)**

- 3- Plaque formation:** Plaques are areas of virally-infected cells in a monolayer cell culture. They are seen by the naked eye as unstained areas when using a vital dye (Fig. 65).



**Fig. (65): Plaque formation**

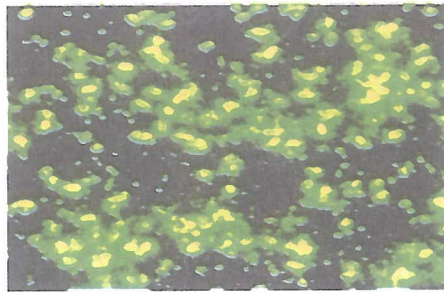
- 4- Transformation:** The nucleic acid of the virus gets incorporated with the genetic material of the cell resulting in cell transformation e.g. in case of herpes virus and other oncogenic viruses. This could be detected as foci of malignant cells in the cell line used.
- 5- Haemadsorption:** Haemagglutinating viruses, e.g. influenza viruses, are detected by adding a suspension of RBCs to the infected cell line. The RBCs will adsorb only to the virally-infected cells (Fig. 66)



**Fig. (66): Haemadsorption**

**6- Interference phenomenon:** Some viruses, e.g. rubella virus, produce no change in cell culture. The presence of the virus could be detected by its ability to interfere with the growth of another CPE-producing virus added as an indicator.

**7- Direct fluorescent antibody staining of infected cells (DFA) (Fig. 67)**



**Fig. (67): (DFA)**

**8- Detection of viral antigens** released in the growth medium can be done by one of the serological tests.

**9- Neutralization test:** This can be done by the use of specific antiviral antibodies which will block or neutralize the infectivity of the virus.

### **b- Embryonated eggs**

Eggs contain a variety of membranes that support multiplication of certain viruses.

### **c- Laboratory animals**

This was the first method used for cultivation of viruses. It is still used mainly for research.

## **B- Indirect Methods:**

### **I. Serological diagnosis**

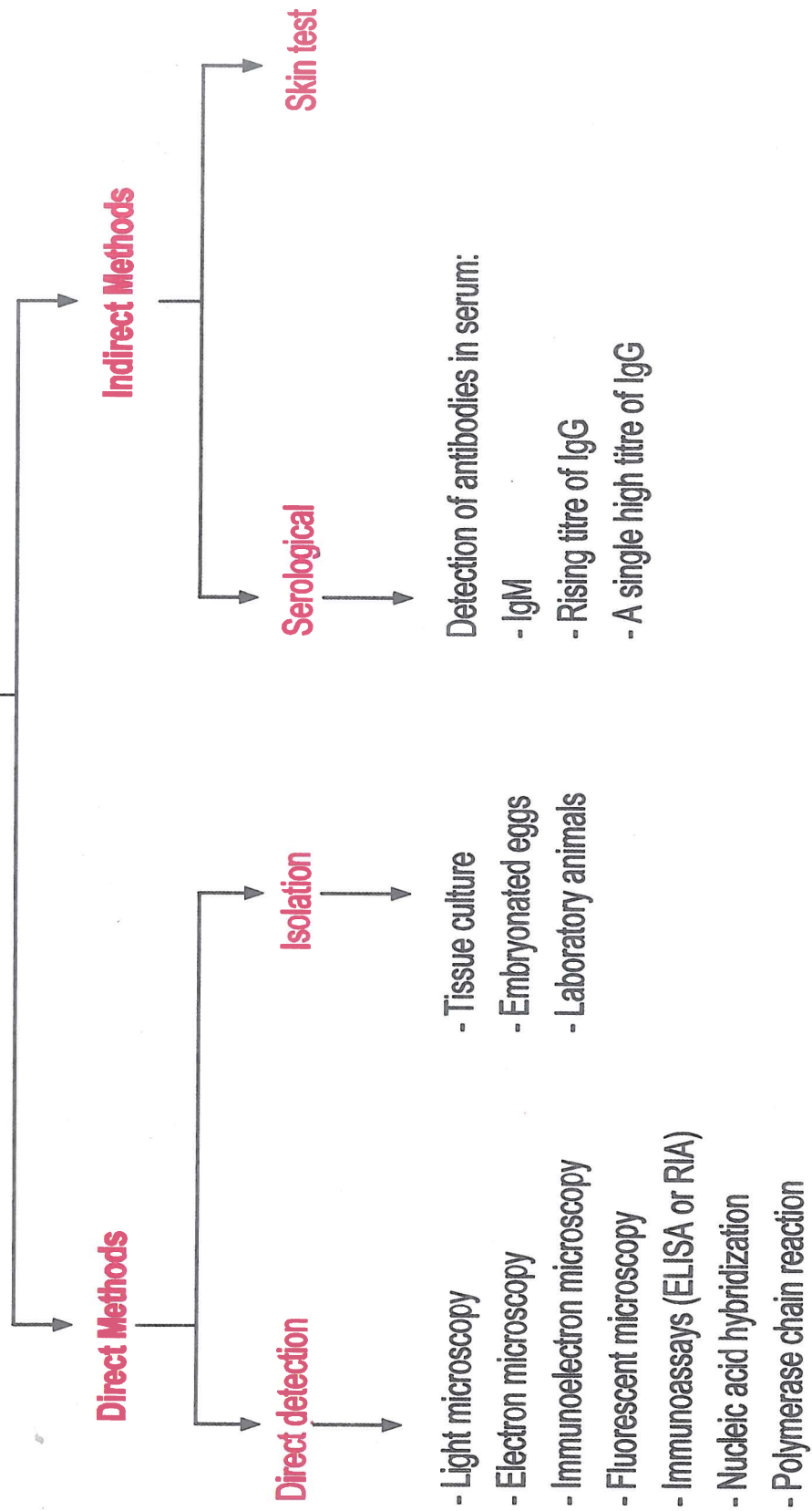
- Detection of antiviral antibodies is an indirect for diagnosis.
- Usually 2 serum samples (paired serum) are taken. The first in the acute phase and the second 2-3 weeks later, to demonstrate a rising titre (4 fold increase or more is diagnostic).
- Only one sample may be used in the acute stage to detect IgM, e.g. in diagnosis of rubella in early pregnancy.

The serological methods used are: neutralization test (NT), complement fixation test (CF), haemagglutination inhibition test (HI), enzyme-linked immunosorbant assay (ELISA), radioimmunoassay (RIA) and indirect immunofluorescence (see Chapter 3).

**II. Skin tests** can be used as an indication of cell-mediated immunity (CMI) in some viral infections.

The choice will vary according to the suspected virus.

## Diagnosis of viral infections





## (C) Fungal Infections

The laboratory diagnosis of fungal infection involves 2 main diagnostic methods:

**A- Direct methods:** which depend either on the detection of fungi and/or their antigens in the patient's specimens, or on isolation of fungi.

**B- Indirect methods:** which depend on detection of serum antibodies against the suspected fungus in systemic mycosis or, less frequently, on skin tests.

**A. Specimens** are collected according to the site of infection such as skin scales, nail clippings, hair, respiratory secretions, biopsies or blood (Fig. 68).



Fig. (68): Specimens for diagnosis of fungal infections

### B. Direct detection

#### 1- Microscopic examination:

- **Unstained (wet) preparations** are examined to demonstrate hyphae, spores or yeast cells. In case of superficial and cutaneous mycoses, the specimen (skin scales, nail clippings or hair) is first mounted with 10% KOH to dissolve keratin, allowing fungal visualization (Fig. 69).
- **Stained preparations:** Different stains are used, e.g. Gram's stain, India ink and silver stains (Fig. 70).

#### 2- Antigen detection in the specimen (e.g. *Cryptococcus* antigens in CSF).

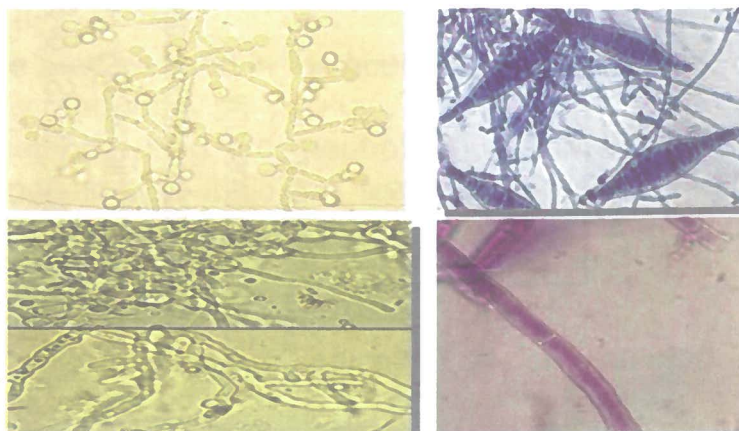
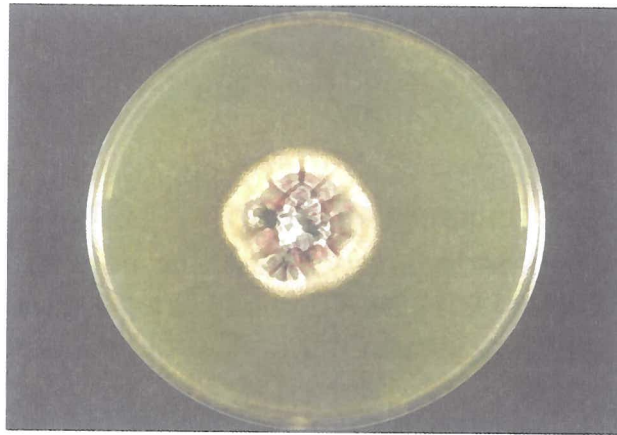


Fig. (69): Unstained smears      Fig. (70): Stained smears

### C. Cultivation:

- Sabouraud's dextrose agar (SDA) is the most commonly used medium (Fig. 71).

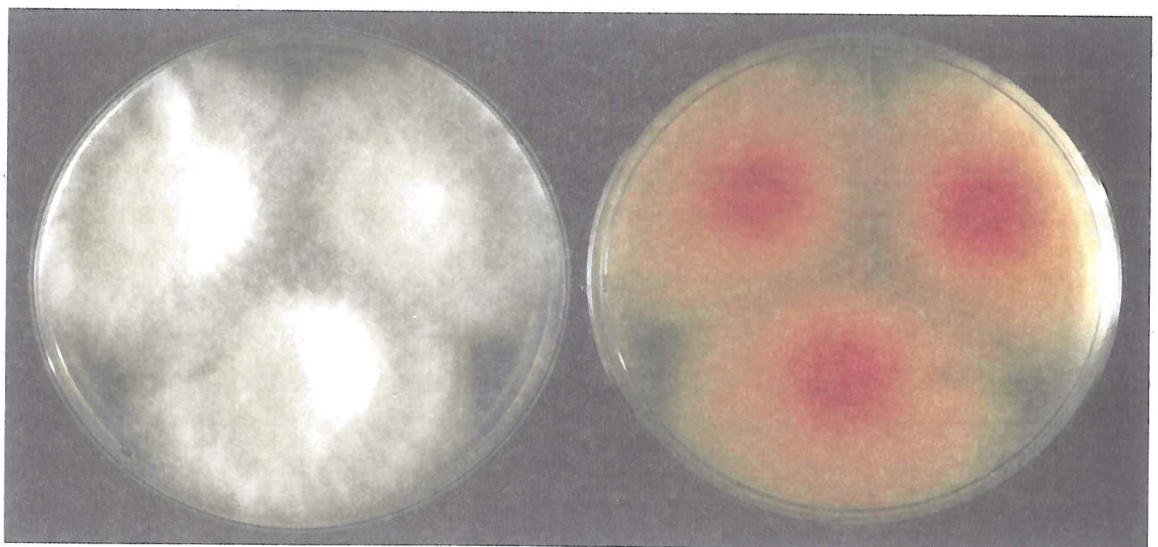


**Fig. (71): Fungal growth on Sabouraud's dextrose agar (SDA)**

- Growth of most fungi is better at 25-30°C.
- Growth may take several weeks.
- If deep mycotic infection is suspected (which is usually caused by dimorphic fungi), enriched media are inoculated and incubated at 37°C to allow growth of the yeasty phase of the fungus.

### D. Identification of culture growth by:

- 1- Colony morphology including the surface and reverse views of the growth is the principal way for identifying fungi (Fig. 72).



**Fig. (72): Surface and reverse views of fungal growth**

- 2- Microscopic examination of wet and stained smears to distinguish the different types of hyphae and conidia (spores).
- 3- Biochemical tests.
- 4- DNA probes can be used to identify fungi growing in culture at earlier stage when the colony is much smaller.

**E. Serodiagnosis:**

Detection of specific antibody may help in the diagnosis of systemic mycoses.

**F. Skin testing** (delayed type hypersensitivity).



## Chapter 3

# ANTIGEN-ANTIBODY INTERACTION

*In vitro* antigen-antibody reactions (i.e. serologic reactions) provide methods for the diagnosis of diseases and for identification and quantitation of antigens and antibodies.

### Characters of Antigen-Antibody Reactions

1. A reaction between antigen and antibody is **specific**, i.e. an antibody can combine only with the antigen which induced its formation. So, an unknown antigen (or antibody) can be identified by reacting with the known antibody (or antigen).
2. The same antibody reacting with the same antigen under different conditions may give **different observable results**. For example, antibodies against pneumococcal capsule reacting with capsulated pneumococcus would give rise to agglutination, while the same antibody reacting with the purified soluble capsule extract will lead to precipitation. Also, if RBCs are mixed with their antibodies, clumping of RBCs (agglutination) will occur. If complement is added to this reaction, lysis of the red cells will occur. So, the same antigen-antibody system can give agglutination in one condition and lysis in another condition. Thus precipitation, agglutination, lysis and other observable reactions reflect types of reactions rather than types of antibodies.
3. Serologic reactions can be performed not only **qualitatively** to determine whether antibodies are present or not, but also **quantitatively** to determine the amount of antibodies present. This is done by preparing different dilutions of the patient's serum, e.g. 1/10, 1/20, 1/40 ... etc. and then mixing each dilution separately with a constant amount of known antigen suspension. The highest dilution (lowest concentration) of the patient's serum giving positive reaction is called the "**antibody titre**".  
For the diagnosis of infectious diseases, two serum samples separated by 7-10 days interval should be tested. A **rising antibody titre** of four-fold (two dilutions) or more means that the antibody response is increasing with progress of illness. This indicates active (ongoing) infection.
4. **Zone phenomenon:** (Fig. 73)  
Observable union between antigen and antibody occurs best when both reactants are present in optimal proportions. No visible reaction occurs in the presence of antigen excess or antibody excess.  
If a series of tubes is set up, each containing a constant amount of antigen, and decreasing amounts of antibody are added to the tubes in the row, a reaction

will start to appear in the tubes, gradually increasing along the row, reaching a maximum, and then falling off with the lower antibody concentration. In the first few tubes of the dilution series, small soluble complexes are formed due to presence of antibody excess, while small soluble complexes are formed in the last few tubes of the dilution series due to presence of antigen excess. In between, visible large aggregates occur.

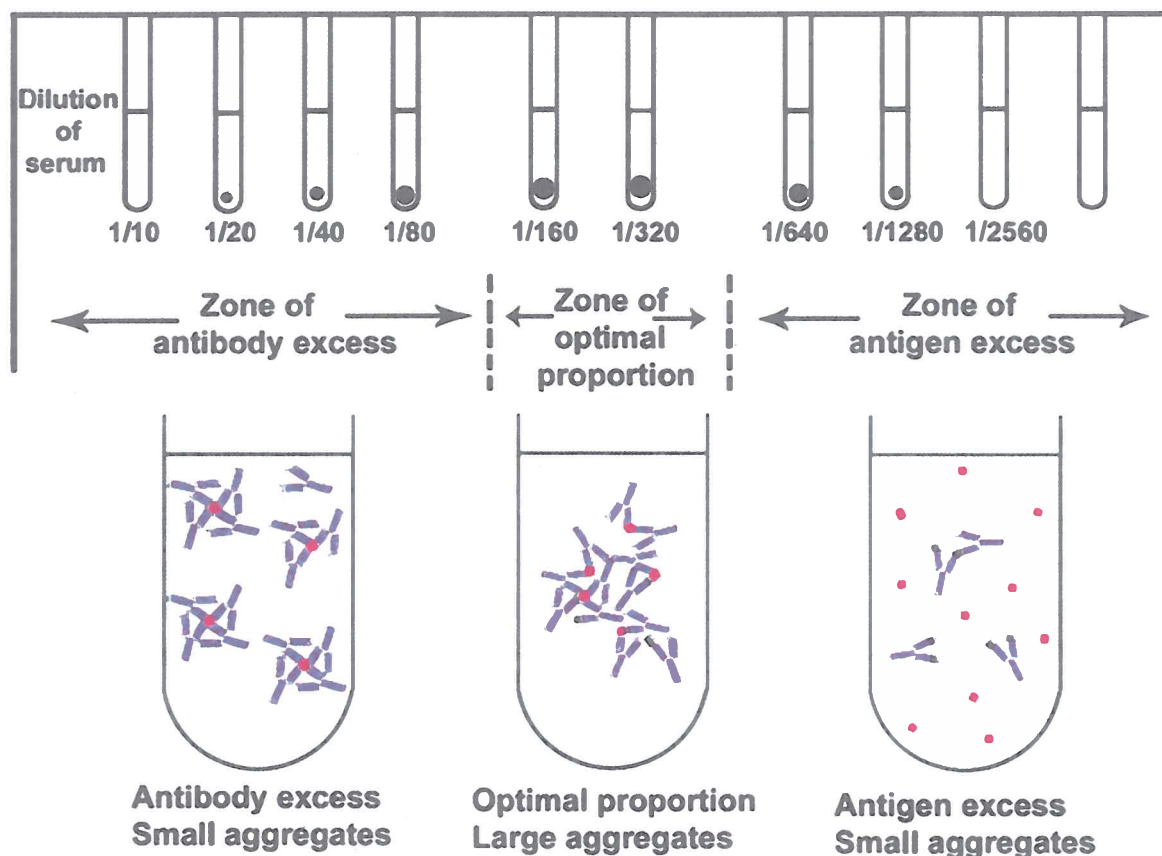


Fig. (73): Zone phenomenon

- The nature of the reaction between antigen and its antibody is mainly physical involving electrostatic forces. It generally requires the presence of electrolytes, e.g. NaCl, and a suitable pH around neutrality. It can occur anywhere between 4°C and 55°C, but certain antigen-antibody systems require certain temperatures for optimal reactions to take place.

## Methods of Detection of Antigen-Antibody Reactions

### I. Reactions Accompanied by Visible Phenomena

In these reactions, the resulting antigen-antibody complexes can be seen either by the naked eye or under the microscope.



## A- Agglutination

A reaction between antibody and its antigen will result in agglutination (clumping) if the antigen is **particulate** (Fig. 74). Under these conditions, the antigen is called "agglutino-gen" and the antibody "agglutinin".

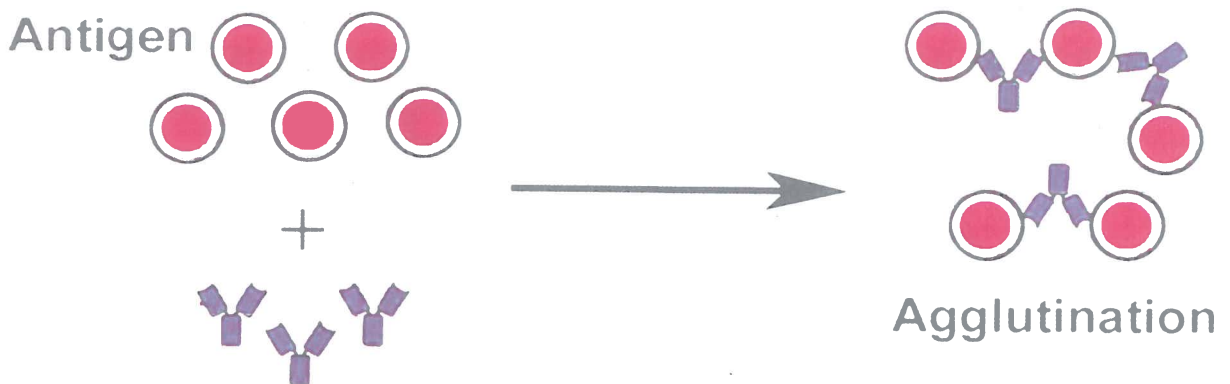


Fig. (74): Agglutination

Agglutination tests have many clinical applications in medical practice. Generally speaking, since the reaction between agglutino-gen and agglutinin is specific, therefore, if one of them is unknown we can identify it with the help of the other.

Agglutination reactions can be performed by 2 different ways:

### 1. Slide Agglutination (Qualitative Test) (Fig. 75)

This test is used to detect or verify the presence of an unknown antigen by means of a known antiserum. Thus, the exact antigenic type of a red cell or bacterium can be identified with standard specific antiserum. A drop of particle suspension (antigen) and a drop of antiserum (antibody) are mixed on the slide and the slide is gently rocked for few minutes; clumping will be observed in positive cases (if antiserum is specific for the antigen).

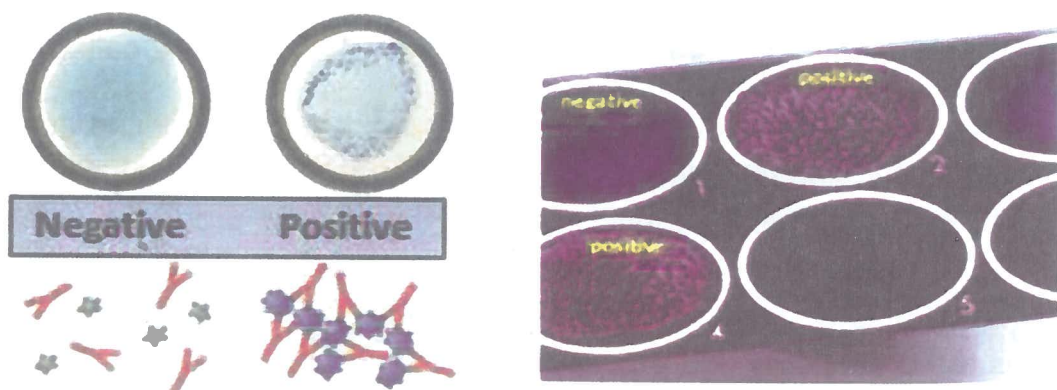


Fig. (75): Slide agglutination test



## 2. Tube Agglutination (Quantitative Test) (Fig. 76)

This test is commonly used in the serological diagnosis of infectious diseases, i.e. detection of antibodies against a certain pathogen; e.g. a patient suspected on clinical grounds to be suffering from typhoid fever would be expected to have high level of antibodies against *Salmonella Typhi* in his serum by the second week of illness. His serum is taken (unknown antibody) and is mixed with a suspension of known typhoid bacillus. If agglutination occurs, this can be taken as indirect evidence for the diagnosis of typhoid fever. The test is usually performed quantitatively by using serial dilutions of the patient's serum to determine antibody titer.

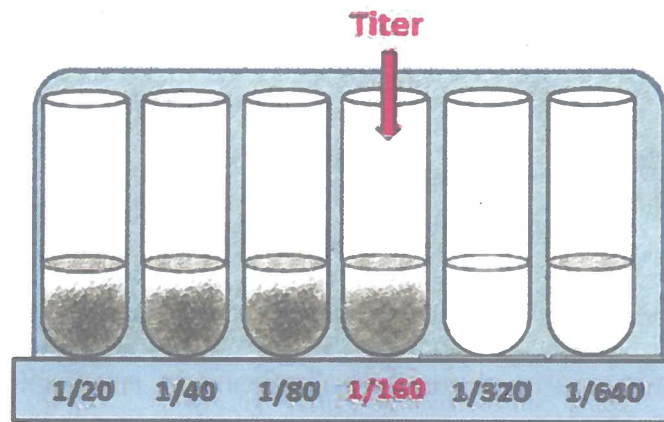


Fig. (76): Tube agglutination test

## B- Passive Agglutination

It is the conversion of a reaction system that results in precipitation to one that results in agglutination, thus yielding a more sensitive indication of antibody or antigen.

This is done by adsorbing the known reactant (soluble antigen or antibody) passively on the surface of inert particles (e.g. latex particles or RBCs); thus either the antigen or the antibody will become particulate resulting in agglutination instead of precipitation (Fig. 77).

If RBCs are used as inert particles, the reaction is called passive haemagglutination.

Examples of passive agglutination tests include:

- 1- Diagnosis of rheumatoid arthritis in which the patient produces an antibody (mainly IgM) to his own IgG. This antibody is called **rheumatoid factor (RF)**. Agglutination of latex particles coated with IgG when mixed with patient's serum indicates the presence of RF (positive test).

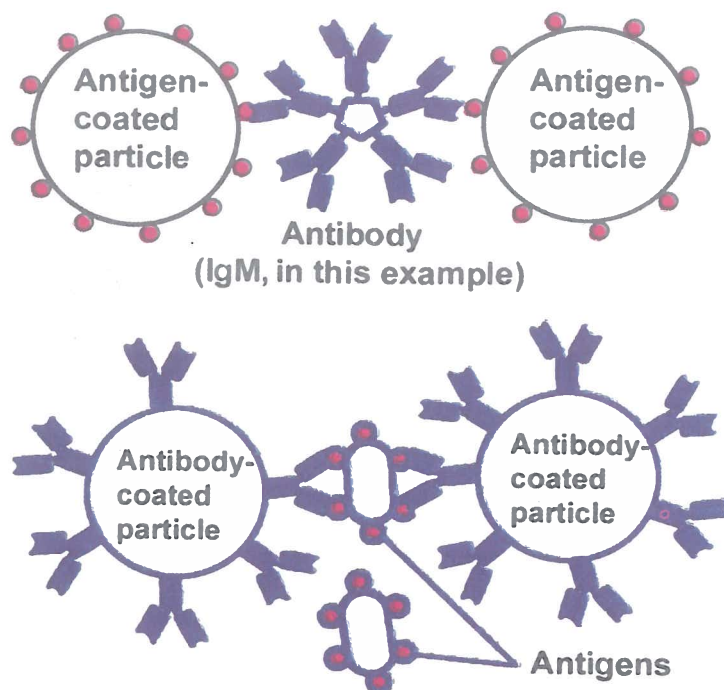


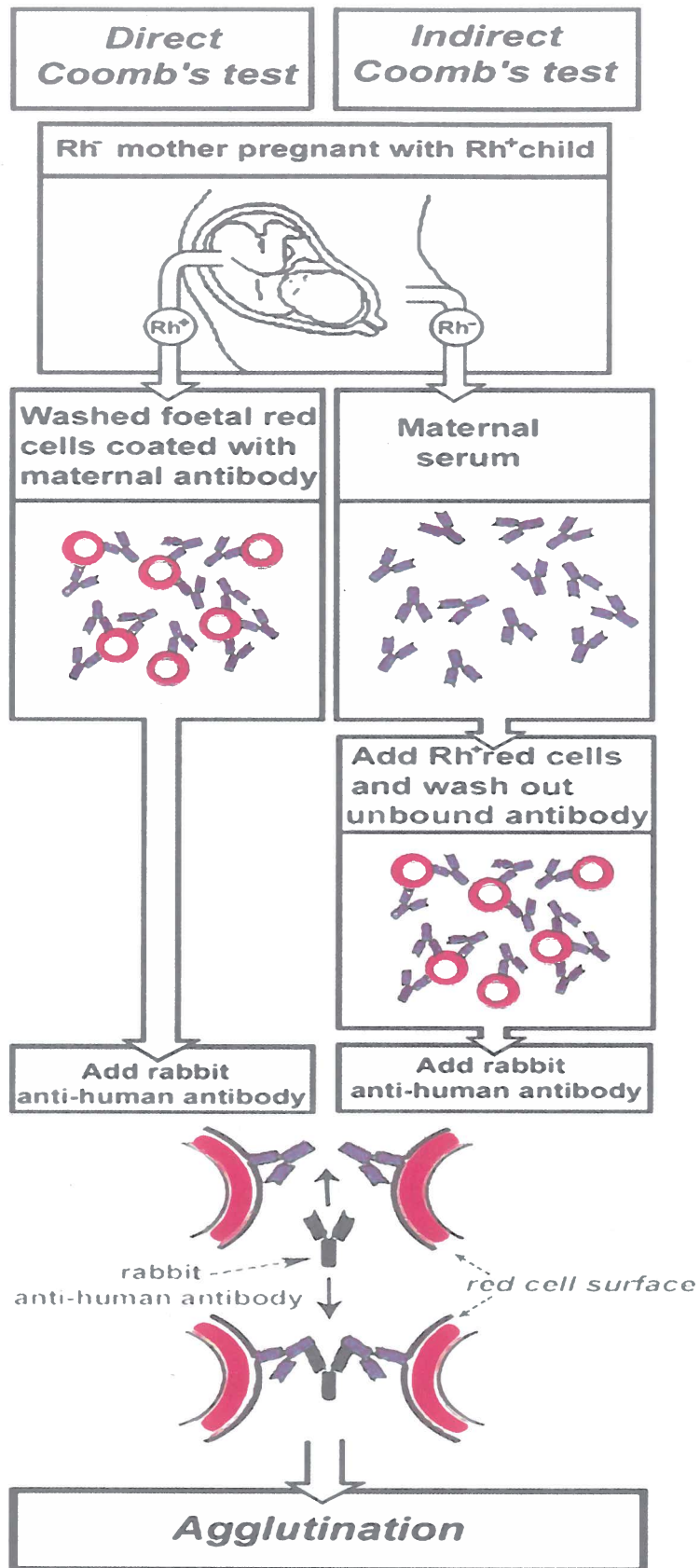
Fig. (77): Passive agglutination.

- 2- Detection of **C-reactive protein** as an indicator of inflammation. Serum of patients agglutinate latex particles coated with anti C-reactive protein antibody.
- 3- **Immunologic pregnancy test** where latex particles coated with anti-HCG (human chorionic gonadotrophic hormones) are mixed with a drop of urine. Agglutination occurs if HCG is present in urine (positive pregnancy test).
- 4- Diagnosis of **rheumatic fever** (a post-streptococcal disease) is done by detecting antibodies to streptolysin O toxin; these are formed in response to infection with *Strept. pyogenes*. The patient's serum is added to latex particles coated with streptolysin O toxin. Agglutination denotes presence of antistreptolysin O (ASO) antibodies. The test is done quantitatively by using serial dilutions of the serum to determine ASO antibody titer (up to 200 units is considered normal).

### C- Coombs' (Antiglobulin) Test

Sometimes antibodies to red cell antigens react with the cell surface but can not bridge between two RBCs to give direct agglutination (non-agglutinating antibodies). This occurs commonly with anti-Rh antibodies and autoimmune haemolytic anaemia.

It is, however, possible to show that the red cells are coated with antibody by adding an anti-human globulin (antibody against human immunoglobulin) which will bring about agglutination of the cells. This is the basis of Coombs' test which is performed in two ways (Fig. 78):



**Fig. (78): Coombs' test.**



## 1. Direct Coombs' Test

This test is performed to detect non-agglutinating antibodies coating the red cells of **newborns** with erythroblastosis foetalis or patients with autoimmune haemolytic anaemia. The patient's RBCs are first washed and the antihuman globulin is added to the cell suspension. Agglutination occurs in positive cases.

## 2. Indirect Coombs' Test

This test is done to detect circulating non-agglutinating antibodies in the serum of Rh negative **mothers** sensitized with Rh antigen. The serum sample is added to Rh-positive, group O red cells and incubated. After washing, antihuman globulin is added. Non-agglutinating antibodies (if present in the patient's serum) will bind to the red cell surface and the antiglobulin will link them together giving visible agglutination.

## D- Haemagglutination Inhibition Test

Haemagglutination involves the agglutination of red blood cells by antibodies (i.e. haemagglutinins), certain virus particles (e.g. influenza and mumps viruses), or other substances.

The ability of certain viruses to cause haemagglutination can be used for diagnosis of such viral diseases, since specific anti-viral antibodies bind to the viral particles and block their ability to agglutinate RBCs (Fig. 79)

For example, if a patient is suspected of having influenza, his serum is examined for specific antibodies. Serum is mixed with known influenza virus and red blood cells:

a- If antibody is present, haemagglutination will be inhibited.

Antiviral antibody + virus + red blood cells = No Haemagglutination.

b- If no antibody is present, haemagglutination will occur:

Virus + red blood cells = Haemagglutination.

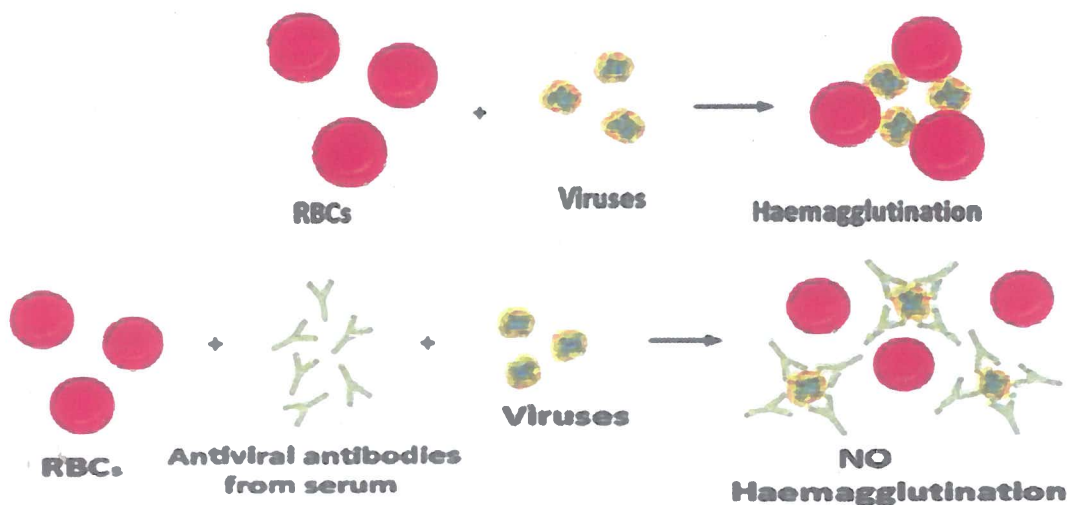


Fig. (79): Haemagglutination inhibition test

## E- Precipitation

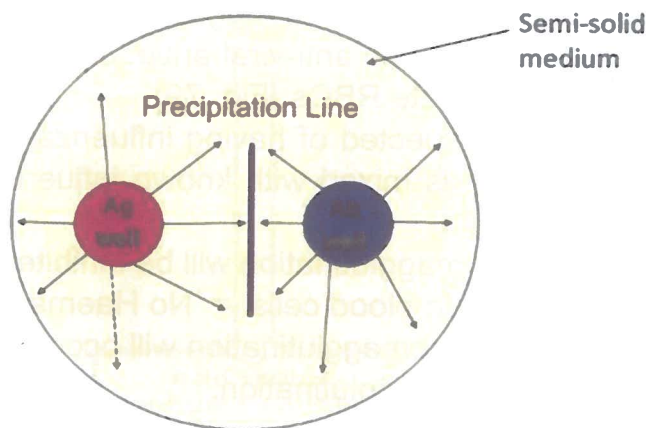
Precipitation occurs when the antigen is soluble instead of cellular; therefore, a large number of molecules are required for lattice formation, and a large lattice must be formed for an aggregate to be visible.

### Applications:

**Agar gel diffusion:** is a commonly used technique, in which antigen-antibody reaction takes place in semisolid media (e.g. agar). It can be performed in 2 different ways:

#### a- Double diffusion

1. Antigen and antibody preparations can be placed in separate wells that are cut in a thin layer of agar in a Petri-dish. The reactants diffuse towards each other through the agar. Where they meet at optimal proportions, bands of precipitate are formed (Fig. 80).



**Double Immunodiffusion**

**Fig. (80): Double immunodiffusion**

#### 2. Elek's test:

- This test is used for demonstrating the toxigenicity of an isolated strain of *C. diphtheriae* (Fig. 81).
- The strain tested is streaked on the surface of a serum agar plate. A strip of filter paper soaked with antitoxin is placed perpendicular to the organism.
- After incubation, the appearance of precipitation lines at the angles indicates toxigenicity of the strain (lines occur where the toxin and antitoxin meet at optimum proportions).

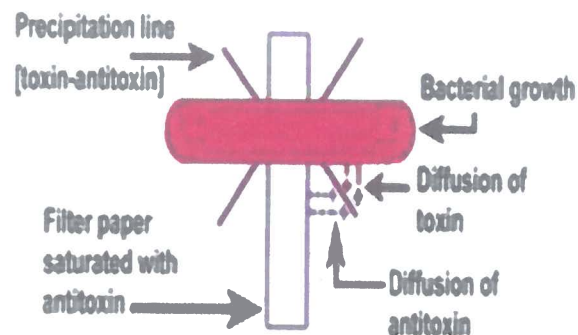
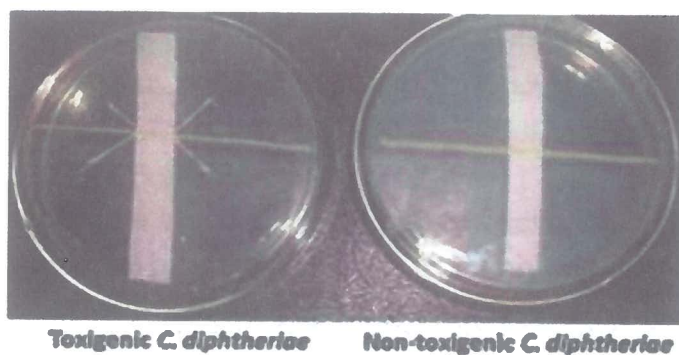


Fig. (81): Elek's test.

#### b- Single radial immunodiffusion

- This is used to quantitate soluble antigens. In this technique, the antibody is mixed with the agar (before pouring it in the plates), and the antigen is placed in wells punched in the agar gel.
- The antigen diffuses radially from the well until a point is reached where its concentration is optimal for precipitation to occur.
- Precipitation will show up as a ring around the antigen well. The diameter of the ring is proportional to the concentration of the antigen in the well (the greater the initial concentration of the antigen in the well, the further it must diffuse to reach the state of optimal proportions with the antibody in the gel) (Fig. 82).
- This technique is routinely used to quantitate various immunoglobulin classes in human serum samples, e.g. quantitation of IgG (antigen of unknown concentration) by using agar gel mixed with anti-IgG antibody.

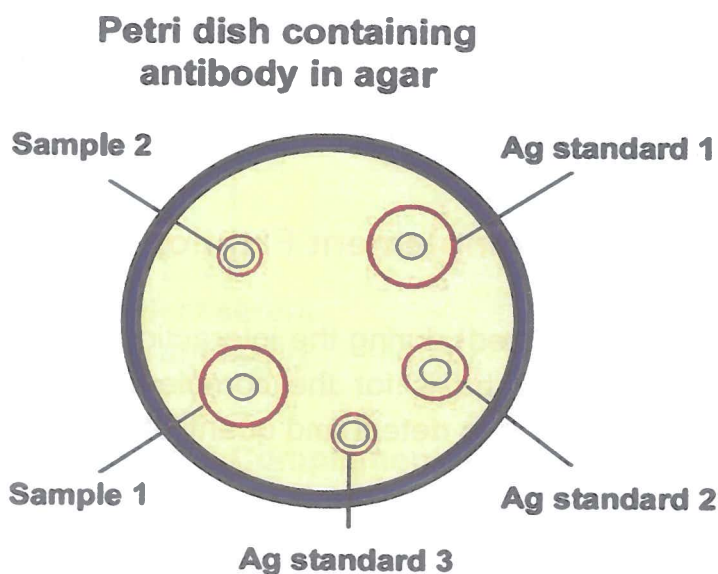


Fig. (82): Single radial immunodiffusion.



## F- Flocculation

- This is another form of antigen-antibody reaction that occurs if the antigen is neither cellular nor soluble, but is a small insoluble particulate antigen.
- The **Venereal Disease Research Laboratory (VDRL) test** is a slide flocculation test used for the serological diagnosis of syphilis. Instead of using treponemal antigen (which is difficult to obtain), the antigen used is water-insoluble cardiolipin. This forms microscopical aggregates in the presence of a heterophil antibody called reagin that is found in the serum of patients with syphilis (Fig.83).
- In the **rapid plasma reagin test (RPR)**, finely divided carbon particles are added (to enable the result to be read by naked eye) (Fig.84).

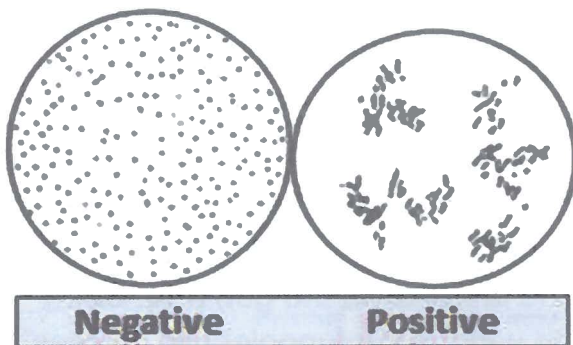


Fig. (83): VDRL test (flocculation test)

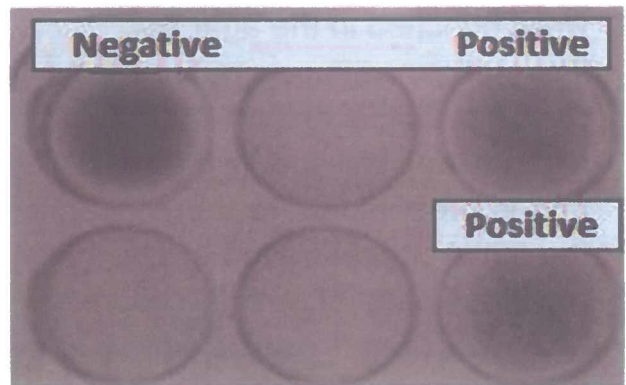


Fig. (84): RPR test (flocculation test)

## G- Complement Fixation Test

Complement is consumed (fixed) during the interaction of antigens and antibodies. This phenomenon forms the basis for the complement fixation test, which is a sensitive test that can be used to detect and quantitate antigens and antibodies.

Guinea pig serum, being rich in complement, is used in the laboratory as a source for complement.

Two systems are used in complement fixation test: (Fig. 85)

**a- Test system:** In the first step, the serum, which is heated to 56°C to inactivate native complement, is added to measured amounts of antigen and complement. If antibody specific for the known antigen is present in the serum, antigen-antibody complexes will form that consume (fix) all the complement. This initial reaction, however, cannot be seen.

**b- Indicator system:** In the second step, an indicator system consisting of sheep red blood cells (SRBCs) plus haemolysin (an antibody specific for SRBCs) is added to test for the presence of free complement.

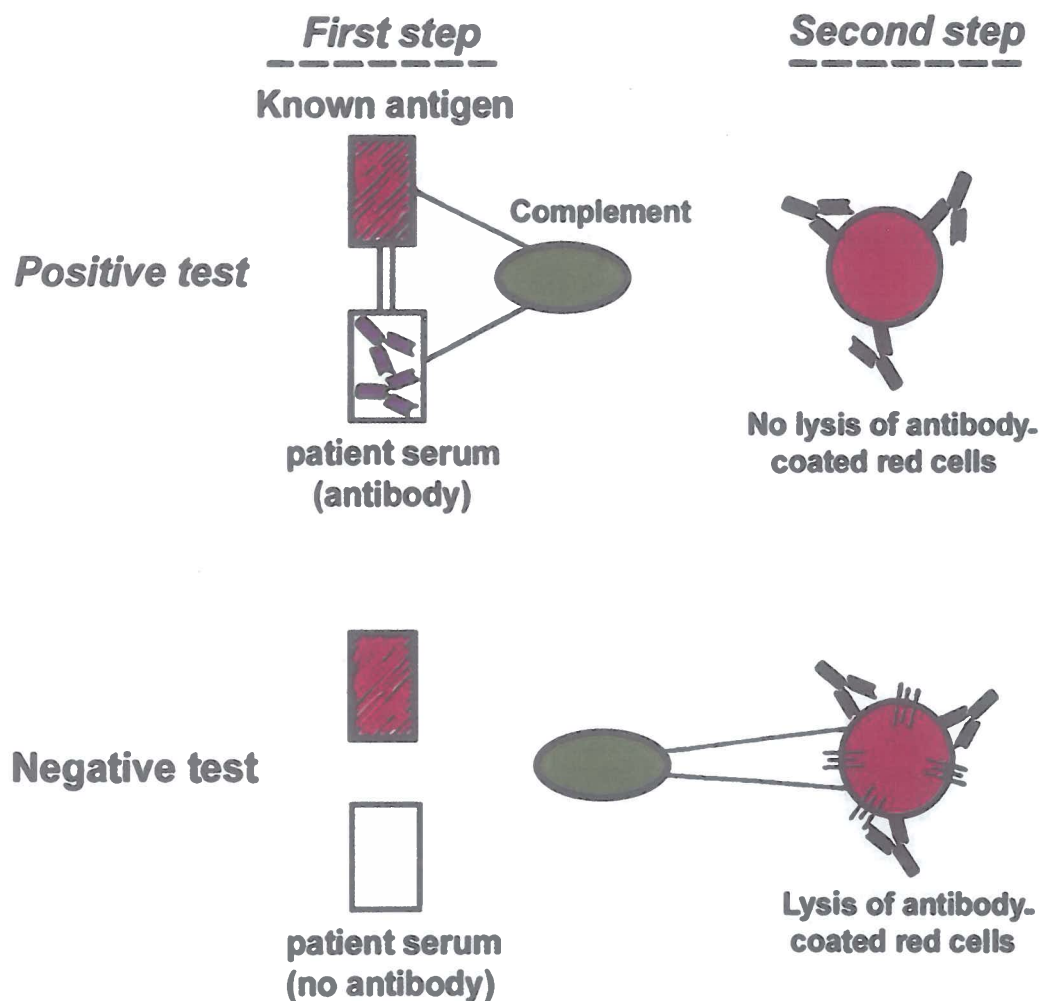


Fig. (85): Complement fixation test

Interpretation of the test is based on the presence of haemolysis:

- If antibody is present in the patient's serum, all the complement will be fixed, and none will be free to lyse the SRBCs. This constitutes a **positive complement fixation test** (No Haemolysis = +ve test).
- If no antibody is present in the patient's serum, then the complement is not fixed and is free to interact in the indicator system and lyse the SRBCs. This constitutes a **negative complement fixation test** (Haemolysis = -ve test).

Complement fixation tests are used in the diagnosis of different infectious diseases.

## H- Virus Neutralization

Certain viruses, when added to appropriate cell culture, produce observable cell destruction referred to as **cytopathogenic effects (CPE)** (Fig. 86) These effects can be inhibited by virus-neutralizing antibodies.

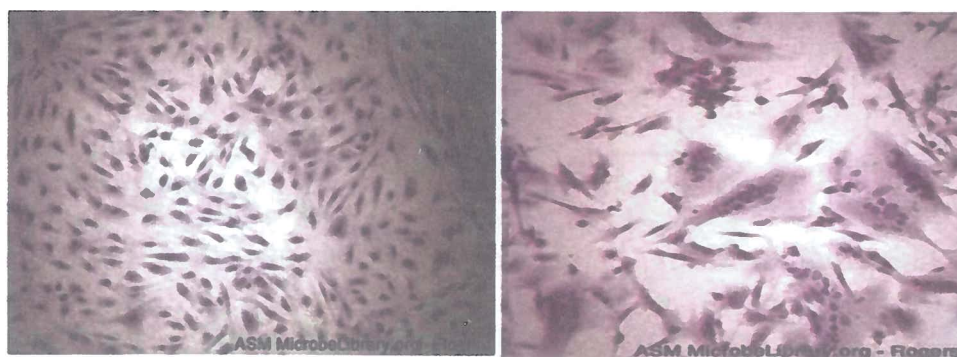
The phenomenon of CPE is useful in the search for virus-neutralizing antibodies in a serum sample. The serum suspected of containing antibody is added to a known virus suspension, and then a susceptible cell culture is inoculated with the mixture.

a- If no CPE develop, then antibodies were present in the serum sample.

Serum antibody + virus + target cell = no CPE.

b- If CPE develop, then no neutralizing antibodies were present:

Virus + target cell = CPE.



**No CPE**  
**(Ab present in serum)**

**CPE**  
**(No Ab in serum)**

**Fig. (86): Viral Neutralization test**



## II. Reactions in which Antigen-Antibody Complexes are Detected by Labelled Reagents

### A- Immunofluorescent Techniques

Fluorescent substances (fluorochromes), such as fluorescein can be conjugated to antibody molecules to allow visualization of the molecules under ultraviolet light, using a fluorescence (UV) microscope (Fig. 87). Such labelled antibodies may then be used to identify antigens.

Both direct and indirect techniques are available (Fig. 88).

#### 1. Direct Immunofluorescence: (Fig. 88a)

- It is used to detect presence of a certain antigen in tissue sections fixed on a microscopic slide.
- Specific fluorescein-labelled antibody is used. If the antigen is present, the antibody will bind to it and will be visualized as green fluorescence on the specimen when it is examined under ultraviolet light using the fluorescent microscope.
- This can be used, for example, in diagnosis of rabies in brain of rabid animals.

#### 2. Indirect Immunofluorescence: (Fig. 88b)

- This is usually employed to detect presence of antibodies against a certain organism in patient's serum using known antigen fixed on a microscopic slide.
- For example, in diagnosis of syphilis the patient's serum (unknown antibody) is allowed to react with *Treponema pallidum* (known antigen) on a slide. After washing to remove unbound antibodies, the slide is overlaid with fluorescein-labelled antihuman globulin. This will bind to the antibody complexed to its antigen and will emit green fluorescence when examined by UV light using the fluorescent microscope.

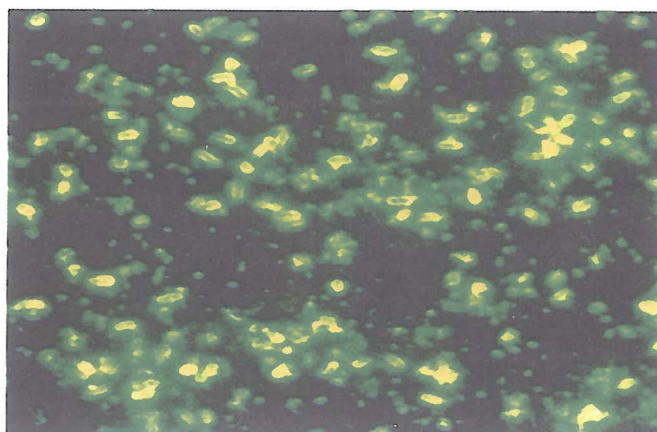
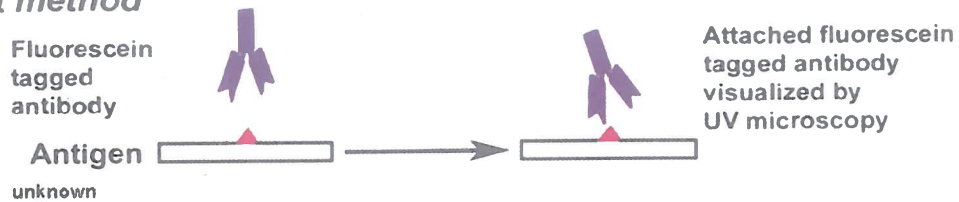


Fig. (87): Positive immunofluorescence

### a) Direct method



### b) Indirect method

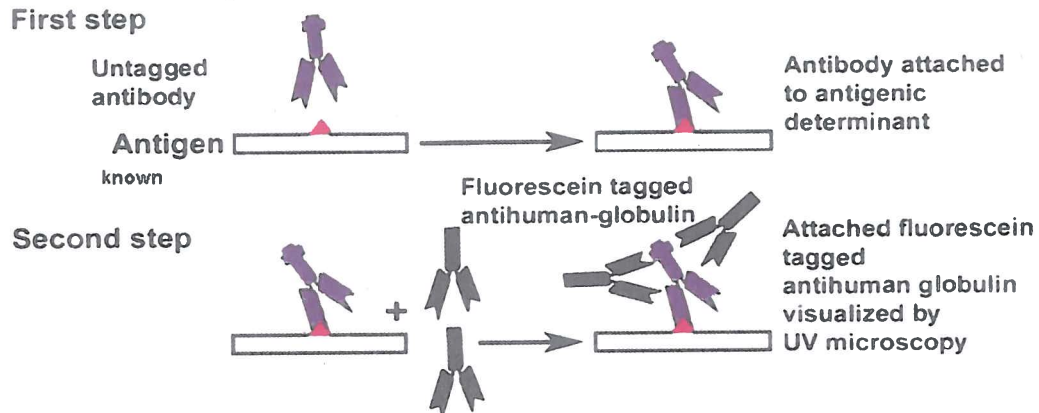


Fig. (88): Immunofluorescent technique

## B- Enzyme-Linked Immunosorbent Assay (ELISA)

- ELISA is a highly sensitive and specific technique for the **detection and quantitation** of antigen and antibody.
- In ELISA, antigens or antibodies are conjugated to an enzyme (e.g. alkaline phosphatase). The resulting conjugate is then both immunologically and enzymatically active.
- The presence of the antigen-enzyme or antibody-enzyme complex can be detected by addition of a proper colourless substrate, which, in presence of the enzyme, becomes converted into a coloured product.
- The degree of colour change, can be measured spectrophotometrically, and is considered as an indicator for the amount of antigen or antibody in the sample (Fig. 89).

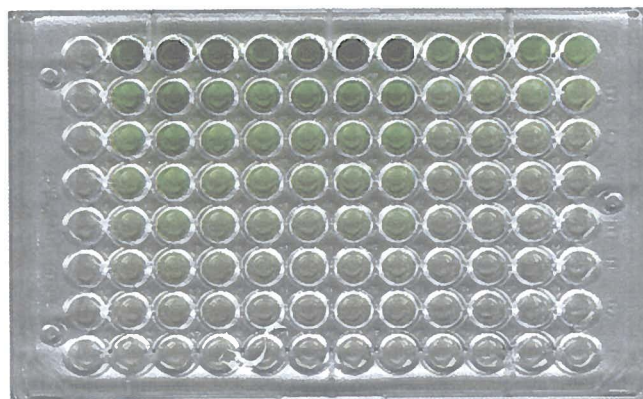


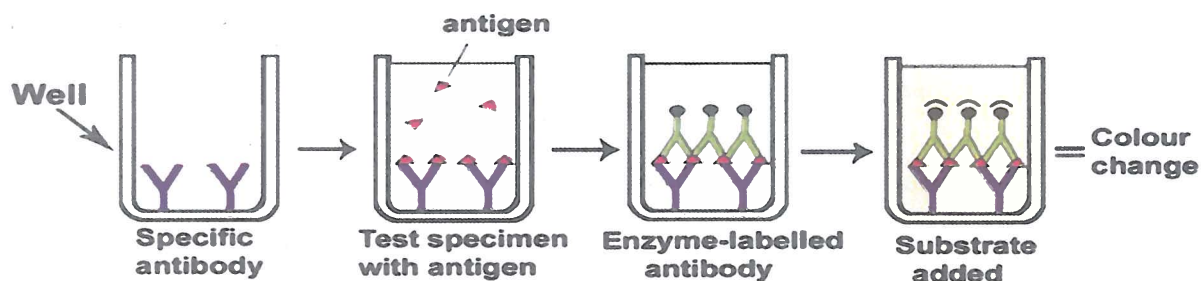
Fig. (89): ELISA plate



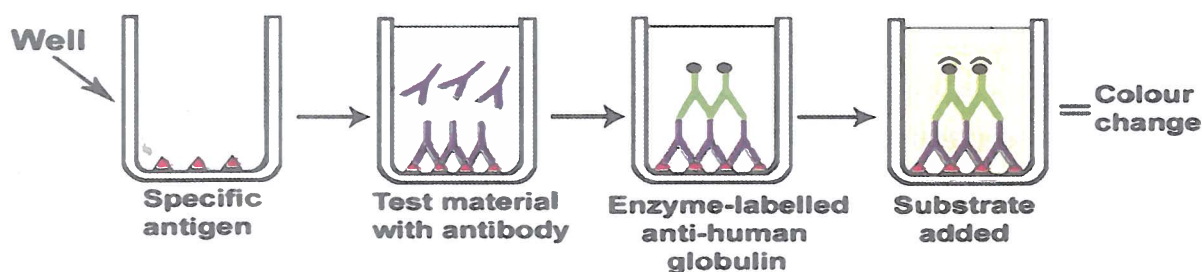
There are a number of variations of the technique. The following are 2 examples:

- For the assay of an antigen, the **double antibody technique** (direct method) is used (Fig. 90a):
  - Known specific antibody is immobilized by adsorption onto a plastic surface.
  - The clinical sample tested for presence of the antigen is added. If antigen is present, it will bind to the immobilized antibody.
  - Any unbound material is removed by washing.
  - An enzyme-labelled specific antibody (conjugate) is then added, which attaches to the fixed antigen.
  - Excess unbound conjugate is removed by washing.
  - Finally, the enzyme substrate is added to detect the presence of enzyme-labelled antibody by the colour produced.
- For the detection of antibody (in serum), **indirect method** is used (Fig. 90b):
  - Known antigens are immobilized by adsorption onto a plastic surface.
  - The test serum is added. If specific antibodies are present, they will bind to the antigens.
  - Non-specific unbound antibodies are removed by washing.
  - An enzyme-labelled anti-human gamma globulin (conjugate) is added, which attaches to the Fc portion of the specific bound antibody.
  - Excess unbound conjugate is removed by washing.
  - The presence of bound conjugate can be detected colourimetrically by adding the substrate for the enzyme.

**a) Direct method**



**b) Indirect method**



**Fig. (90): Enzyme linked immunosorbent assay (ELISA).**



## C- Western Blot (Immunoblot)

The Western blot is primarily used in medicine for confirmation of HIV infection in a patient who is positive by the ELISA test.

Steps of Western blot:

- The antigens of the virus are separated in an electric field and blotted onto a strip of nitrocellulose paper.
- The serum of the patient is then added and anti-HIV antibodies (if present) are allowed to bind to their corresponding viral antigen on the nitrocellulose paper.
- Enzyme-labelled anti-human globulin is added to bind to the previously bound patient's antibodies (if any).
- The enzyme substrate is then added and a visible colour change will be produced which will be seen in the form of coloured bands.

The test is considered reactive (positive) if the patient has antibodies that react with at least two of the following viral proteins: p24, gp41 and gp120 (Fig 91).

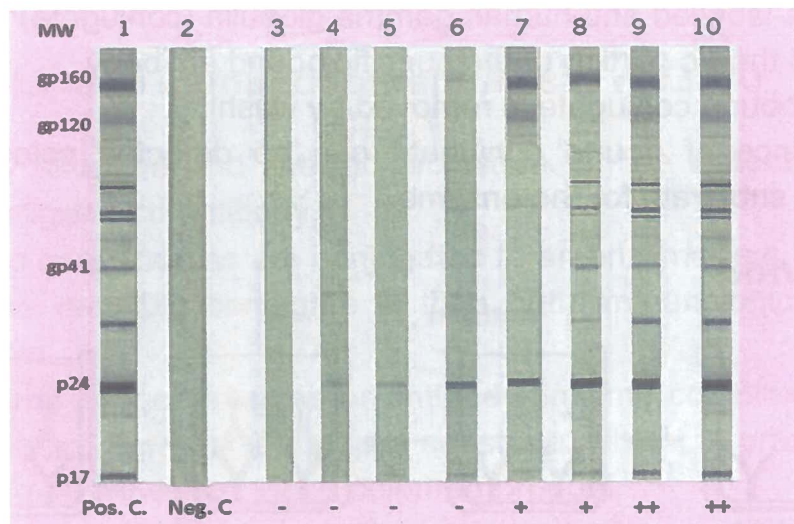


Fig. (91): Western blot for diagnosis of HIV

## D- Radioimmunoassay (RIA)

- In RIA, a radioactive isotope (e.g. iodine<sup>125</sup>) replaces the enzyme in ELISA technique to label antigen or antibody. Antigen-antibody reaction is measured in terms of radioactivity.
- RIA has the same sensitivity as ELISA; however, its use is accompanied by hazards of radiation. As in ELISA, there are several variations of the technique.
- RIA is used to quantitate many biological substances, e.g. hormones, tumour markers, drugs ... etc.